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ROENTGENOGRAPHIC RECORD OF GROWTH OF THE FEMUR OF THE RABBIT

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(Received for publication August 15, 1955)

Felts (1954) has investigated the prenatal development of the human femur using preparations stained with Alizarin Red S and cleared in potassium hydroxide and glycerol. He has expressed growth trends in terms of empirical formulae, and has reviewed some of the earlier literature. Serial roentgenograms were not utilized in his study. By means of a chronological series of roentgenograms showing adjacent ends of the femur and tibia, Watson and Tyndale-Biscoe (1953) determined the age of fusion of the epiphysis with the diaphysis of the tibia of a species of wild rabbit. Their longitudinal study, however, apparently did not include determinations of linear dimensions. Femur measurements in relation to postnatal age of the rabbit as determined from projections on timed series of roentgenograms apparently have not yet been published.

This paper will report increase in actual and relative length and diameter of the femur of unoperated, control extremities of the rabbit in relation to postnatal age, body weight and length of the tibia. The study is based on a chronological series of roentgenograms and supplements a similar study of growth of the calcaneus and tibia.

This investigation was aided financially by the University of Missouri Research Council. The writer is indebted to Dr. M. D. Overholser and the Anatomy Department for help, to Dr. C. M. Waggoner and his staff for x-ray services of the University Hospital, and to Dr. H. B. Latimer, Department of Anatomy, University of Kansas, for valuable criticism. Mr. Cecil Watson and Mr. Paul Dennis provided some assistance in computation.

MATERIALS AND METHODS

The animals and the experimental conditions have been described (Lowrance, 1953, 1953a). Projections of femora of animals of known

postnatal age were made on screen type film and were measured with a vernier caliper graduated to 0.1 mm. In the present study, image measurements have not been corrected for magnified radiographic distortion, because the proximal end of the femur may be variably elevated above the cassette, depending on the inclination of the pelvis at the time a roentgenogram is taken; the greater the angle of inclination, the greater the elevation of the proximal end of the femur. This increased elevation results in a projection on the film which is actually shorter than the bone, but it also produces greater magnified radiographic distortion which gives an apparent increase in length of the bone on projection. Thus the one tendency partly compensates for the other, but the resultant projected image usually is shorter than the bone itself. Under the conditions of this study, even without correction, film measurements of femur lengths on terminal roentgenograms of the chronological series range from 0.5% longer to 7.8% shorter than the corresponding actual bone lengths of sacrificed animals. The film measurements average approximately 3% shorter than the actual bone lengths.

In this study, femur length is the maximum longitudinal measurement of the projected image of the bone on the roentgenogram from the cephalic articular surface of the head to the distal extremity (Bensley, 1938). Similarly, femur diameter is the maximum transverse measurement of the image on the film taken at the boundary between the proximal and middle third of the bone in postero-anterior-lateral projection.

Separate arrays of linear values based on the fixed, experimental, chronological schedule and also on the data regrouped in class intervals for each 200 grams increase in body weight were studied statistically. The mean, standard deviation, coefficient of variation, and the probable errors of the mean and coefficient of variation were determined for each array. In addition, significant differences in mean lengths between control and operated extremities, and significant differences in mean lengths and diameters of control extremities in relation to age between males and females were determined by the "t" test (Snedecor, 1946). Empirical, rectilinear formulae describing increases in length and diameter in relation to age and body weight were determined by the method of least squares (Arkin and Colton, 1950).

Results and Comparisons

Differences in lengths and diameters between femora of control and operated extremities are negligible at all ages; the values fall far below the 1% level of significance. Differences in lengths and diameters of control femora of male and female animals are small also, and are not significant at the 1% level for all ages studied (Table 1).

TABLE 1

Mean lengths and diameters of femur, their standard deviations, coefficients of variation; differences between male and female femora of control extremities in relation to age.

AGE IN DAYS	FEMUR LENGTH OF COMBINED SEXES		DIFFERENCE BETWEEN MALE & FEMALE	FEMUR DIAMETER OF COMBINED SEXES		DIFFERENCE BETWEEN MALE & FEMALE
	MEAN & STANDARD DEVIATION	COEFFICIENT OF VARIATION		MEAN & STANDARD DEVIATION	COEFFICIENT OF VARIATION	
	mm.	%	"t"	mm.	%	"t"
15	31.8 ± 3.1	9.7	1.32	3.2 ± 0.4	12.5	0.00
22	38.2 ± 4.2	11.0	0.06	3.8 ± 0.5	13.2	0.46
29	44.8 ± 5.0	11.2	0.16	4.4 ± 0.6	13.6	0.00
36	51.3 ± 3.8	7.4	0.21	5.1 ± 0.7	13.7	0.00
43	55.7 ± 4.4	7.9	0.11	5.6 ± 0.6	10.7	0.84
50	60.0 ± 6.5	10.8	0.12	5.8 ± 0.7	12.1	0.00
57	63.7 ± 4.2	6.6	1.06	6.0 ± 0.6	10.0	0.52
64	68.3 ± 5.2	7.6	1.02	6.3 ± 0.6	9.5	0.00
71	71.9 ± 4.1	5.7	0.39	6.4 ± 0.8	12.5	0.72
78	75.0 ± 3.5	4.7	1.08	6.5 ± 0.7	10.7	0.75
85	76.9 ± 3.3	4.3	0.28	6.7 ± 0.7	10.4	0.00
92	79.0 ± 3.9	4.9	0.28	6.8 ± 0.7	10.3	1.24
106	81.8 ± 5.4	6.6	1.54	6.8 ± 0.6	8.8	0.46
120	85.0 ± 4.7	5.5	1.86	7.0 ± 0.6	8.6	0.45
134	87.1 ± 4.4	5.1	0.34	7.2 ± 0.7	9.7	0.35
148	87.0 ± 4.9	5.6	1.57	7.0 ± 0.7	10.0	0.00
162	88.2 ± 7.0	7.9	0.78	7.1 ± 0.8	11.2	0.71
176	86.9 ± 5.9	6.8	1.53	7.3 ± 0.9	12.3	0.24
190	86.9 ± 4.4	5.1	0.20	7.2 ± 0.9	12.5	0.94
204	87.6 ± 6.0	6.8	0.96	7.3 ± 0.7	9.6	0.00

"t" VALUES OF 2.78 AND ABOVE ARE SIGNIFICANT AT 1%.

For this reason, data from male and female rabbits have been combined.

Mean lengths and diameters of the femora of combined sexes at stated ages are listed in Table 1. These mean values show regular curves of increase in relation to postnatal age in days. In Table 3, formulae 1 through 7 describe these increases in length (L) and diameter (D). Similarly, mean lengths and diameters at stated mean body weights in grams are presented in Table 2, and formulae 8

through 13 of Table 3 describe these regular increases. The average of the mean percentage deviations of the calculated from the means of the observed values does not exceed 0.6% for all empirical formulae listed in Table 3. The formulae closely fit the means of the observed values falling within the age and weight intervals to which the formulae apply.

TABLE 2

Mean lengths and diameters of femur, their standard deviations and coefficients of variation in relation to mean body weight.

MEASUREMENTS OF COMBINED SEXES				
MEAN BODY WEIGHT	MEAN LENGTH & STANDARD DEVIATION	COEFFICIENT OF VARIATION	MEAN DIAMETER & STANDARD DEVIATION	COEFFICIENT OF VARIATION
gms.	mm.	%	mm.	%
165	30.5 ± 2.0	6.6	3.0 ± 0.3	10.0
283	35.8 ± 3.7	10.3	3.5 ± 0.4	11.4
502	44.8 ± 3.3	7.4	4.4 ± 0.4	9.1
686	52.0 ± 3.9	7.5	5.1 ± 0.4	7.8
880	56.1 ± 4.3	7.7	5.4 ± 0.4	7.4
1091	61.1 ± 4.7	7.7	5.8 ± 0.4	6.9
1287	66.9 ± 6.0	9.0	6.0 ± 0.5	8.3
1487	71.9 ± 7.0	9.7	6.1 ± 0.4	6.5
1696	75.7 ± 7.8	10.3	6.4 ± 0.4	6.3
1886	79.8 ± 6.5	8.1	6.4 ± 0.4	6.3
2089	78.8 ± 6.5	8.2	6.7 ± 0.4	6.0
2295	78.9 ± 6.2	7.9	6.8 ± 0.5	7.4
2493	81.5 ± 5.0	6.1	7.0 ± 0.6	8.6
2697	84.3 ± 4.0	4.7	7.1 ± 0.4	5.6
2889	86.1 ± 5.0	5.8	7.3 ± 0.4	5.5
3133	88.3 ± 6.2	7.0	7.5 ± 0.5	6.7
3284	88.7 ± 6.1	6.9	7.8 ± 0.5	6.4
3496	89.0 ± 5.3	6.0	7.6 ± 0.5	6.6
3675	93.4 ± 4.5	4.8	8.2 ± 0.7	8.5

All of the mean values (Tables 1 and 2) exceed their respective probable errors by a factor of 10 or more and therefore are considered to be reliable. Probable errors of the mean and the coefficient of variation are not listed in the tables.

Coefficients of variation in bone lengths and diameters for all age groups studied are listed in Table 1, and similarly, for all mean body weight groups, in Table 2. The mean of the coefficients of variation in length for all age groups is approximately 7%; in diameter for all age groups, 11%. Variation in both length and diameter in relation to age fluctuates, but it tends to be highest in animals younger than 50 days. The animals are growing rapidly between 15 and 43 days, hence greater variation would be expected in this early period. By the fiftieth day which marks the end of the period of most rapid growth,

variation in length decreases and is least in rabbits between 78 and 92 days old; growth in these animals should be expected to be less variable. Variation in length increases again slightly in animals older than 106 days. This later increase could be due in part to prolonged differential slowing of growth in length of the femora of individual animals in the population, especially the smaller ones, preceding visi-

TABLE 3
Empirical formulae describing changes in actual length and diameter of femur in relation to age and mean body weight.

FORMULA NUMBER	EMPIRICAL FORMULAE	RANGE IN WHICH FORMULA IS VALID: POSTNATAL AGE IN DAYS	MEAN PERCENTAGE DEVIATION OF CALCULATED FROM MEANS OF OBSERVED VALUES
	FEMUR LENGTH,		
1	(L), ON AGE = 17.8 + 0.93(DAYS)	15 - 36	0.07
2	(L) = 30.3 + 0.59(DAYS)	36 - 78	0.50
3	(L) = 58.58 + 0.217(DAYS)	78 - 134	0.47
4	(L) = 83.94 + 0.0186(DAYS)	120 - 204	0.72
	FEMUR DIAMETER,		
5	(D), ON AGE = 1.89 + 0.087(DAYS)	15 - 43	0.39
6	(D) = 4.52 + 0.026(DAYS)	43 - 85	0.44
7	(D) = 6.36 + 0.005(DAYS)	85 - 204	1.13
		MEAN BODY WEIGHT IN GRAMS	
	FEMUR LENGTH,		
8	(L), ON BODY WEIGHT = 23.975 + 0.041(GRAMS)	165 - 686	0.47
9	(L) = 35.087 + 0.0243(GRAMS)	686 - 1696	0.74
10	(L) = 62.336 + 0.0081(GRAMS)	1696 - 3675	1.13
	FEMUR DIAMETER,		
11	(D), ON BODY WEIGHT = 2.353 + 0.00403(GRAMS)	165 - 686	0.00
12	(D) = 4.058 + 0.00154(GRAMS)	686 - 1287	0.43
13	(D) = 4.874 + 0.00085(GRAMS)	1287 - 3673	0.83

ble closure of the epiphysial plates. The latter has not occurred in animals of this series at 204 days after birth, but it might occur at about the same time (Washburn, 1946) as closure of the epiphysial line in the knee region of a species of wild rabbit (Watson and Tyndale-Biscoe, 1953); namely, in the period 34 to 44 weeks after birth. Variation in mean diameter of the femur of animals grouped on the basis of age tends to remain high throughout the growth period studied, probably because of the shortness of the dimension and consequent larger error of measurement, and also because of the variable rotation of the femur in different roentgenograms. There is a brief period of minimum variation between 106 and 134 days.

Variation both in length and diameter of femora of animals grouped on the basis of body weight is similar, and is of the order of 7.5%. This is slightly higher than the average coefficient of variation in length in relation to age. Variation both in length and diameter of femora of animals grouped in relation to body weight fluctuates somewhat more erratically than it does in relation to age. This may be due partly to the fact that body weight itself is quite variable, since it is affected by such factors as accumulation of fat, and contents of the digestive tube; and thus weight would accentuate fluctuation in other, dependent variables when it is used as a basis of grouping. Variation tends to be lower on the average in animals heavier than 2493 grams; these animals have passed the period of most rapid growth. Most of the coefficients of variation exceed their probable errors by a factor of 10 or more and therefore are considered to be reliable.

The curves in the lower right part of figure 1 show the mean length and diameter of the femur expressed in terms of the respective length and diameter at 15 days taken as unity and plotted on postnatal age in days. The vertical scale in the left margin applies to these relative length curves. In the period 15 to 43 days, increase in mean relative length and diameter are rapid and very similar. After 43 days, increase in relative length continues with very slight decrease, but increase in relative diameter falls off markedly. By 204 days, the femur has increased in relative length almost 2.8 fold, and in mean relative diameter, approximately 2.2 fold. Even at 204 days there is some evidence of continuing slow growth of the femur in some members of the population, especially in the larger animals, and this may help to explain the increased variation in length in the oldest age groups.

The two upper curves in figure 1 represent the length and diameter relative to the length and diameter of the rabbit 204 days old taken as 100%. Thus the 204 day mean value is considered to be that of the adult measurement. The vertical scale in the right margin applies to these curves. By 15 days after birth, at the beginning of the period of measurement, the femur has attained 32% of its adult length and 44% of its adult diameter. Between 15 and 43 days, the rate of increase in percentage of adult diameter slightly exceeds that of length. After 43 days this rate progressively decreases in comparison

with that of length. Both length and diameter attain approximately 97% of their total increase by 130 days. In this respect the growth of the femur is like that of the calcaneus which also attains 97% of its adult length by 130 days (Lowrance, 1953).

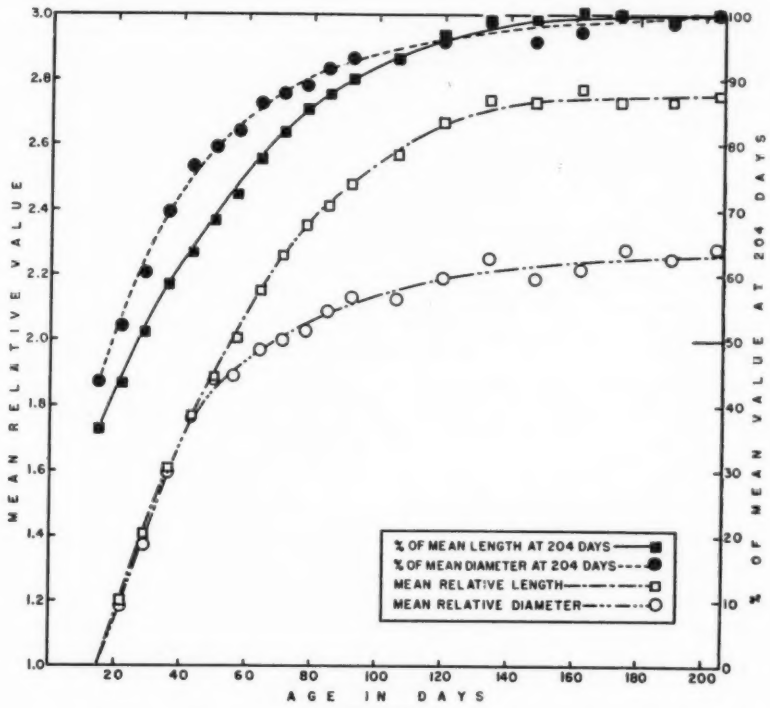
MEAN RELATIVE VALUE
% OF MEAN VALUE AT 204 DAYS
AGE IN DAYS
% OF MEAN LENGTH AT 204 DAYS
% OF MEAN DIAMETER AT 204 DAYS
MEAN RELATIVE LENGTH
MEAN RELATIVE DIAMETER

FIGURE 1

The curves in the lower right portion of the graph with the scale in the left margin show the relation of mean length and diameter in terms of these mean values at 15 days plotted on postnatal age in days. The curves in the upper left portion of the graph with the scale in the right margin show the percentage of mean length and diameter attained at 204 days plotted on age.

In figure 2, mean length of the calcaneus (Lowrance, 1953a), and mean length and diameter of the femur as determined in the present study, have been plotted against mean tibia length (Lowrance, 1953a) on log-log paper. This visual method of showing allometric growth,

applied by Moss (1954, 1955) to linear increase in bone, emphasizes changes in differential growth rates as indicated by changes in slope of the curves. In figure 2, the logarithmic scale in the right margin has 10 times the value of that in the left margin. This arrangement of scales and the placement of the continuous diagonal line in the

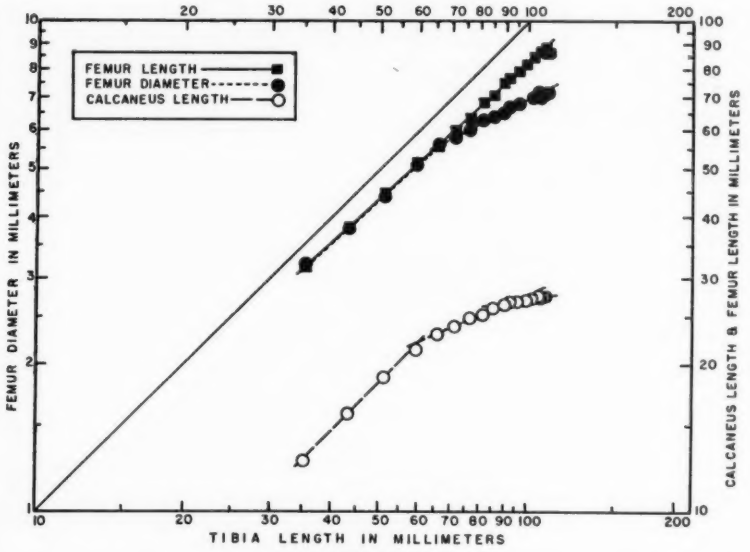

FIGURE 2

Relation of femur length, femur diameter and calcaneus length to tibia length. Femur diameter in millimeters is indicated by the logarithmic scale in the left margin. Femur length and calcaneus length in millimeters is indicated by the logarithmic scale in the right margin.

figure make possible the approximation of all curves and aid the eye in making direct, visual comparisons of the slopes of the curves. These slopes for femur length and diameter are almost identical between tibia lengths 36 and 60 mm. Definite decreases in rates of growth thus occur for calcaneus length at 60 mm and for femur length at 65 mm of tibia length. These changes offer leads for studies on bone growth by other methods. Their significance, if any, should be studied carefully.

Summary

Between 15 and 204 days after birth, in rabbits of this heterogeneous population of combined sexes, the femur increases in mean length from 32 to 88 mm, or almost 2.8 times its length at 15 days. It increases in diameter from 3.2 to 7.3 mm, or approximately 2.2 times its diameter at 15 days. Ninety-seven per cent of both adult length and diameter is attained at approximately 130 days after birth.

Empirical formulae are presented to describe actual increases in length and diameter in relation to age and body weight.

In double logarithmic plots against tibia length, the slopes of the curves of femur length, femur diameter and calcaneus length are very similar from 36 to 60 mm of tibia length. Decreases in slope of the curve for calcaneus length occurs at approximately 60 mm, and for femur diameter at 65 mm of tibia length.

Coefficients of variation fluctuate; those of femur length in relation to age average approximately 7% and those for femur diameter, 11%.

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A STUDY OF LEIOMYOMA, A MESENCHYMAL TUMOUR ON THE FINS OF AN INDIAN CATFISH, *Mystus* (*Osteobagrus*) *seenghala* (Sykes)

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Introduction

The spontaneous occurrence of various types of tumours in lower vertebrates has been reviewed by Schlumberger and Lucké (1948) and it has been shown that tumours of all vertebrates are essentially the same with regard to their structure and behaviour. For some time past we have been studying tumours of fishes and have recorded an epidermal papilloma in *Anabas testudineus* (Sarkar and Dutta-Chaudhuri, 1953). In the present communication we report an interesting case of leiomyoma, with several outgrowths of varying sizes and with peculiar histological pattern occurring in a new site, the fins of *Mystus* (*Osteobagrus*) *seenghala*.

Observations

Morphological: A single healthy and normal specimen of *Seenghala* (standard length 488 mm.) was collected from a local fish market of Delhi. On both the paired and unpaired fins, except the adipose dorsal and pectorals of the fish, twenty-three solid, roughly oval, and glossy cream coloured outgrowths of different sizes (the largest being 26 mm. by 9 mm. and the smallest being 4 mm. by 2 mm.) were observed on the skin either over or in between the fin rays (Fig. 1).

On the dorsal fin three outgrowths were present, two on the left and the remaining one on both the sides. On the left pelvic fin, out of nine growths, five were on the outer and four on the inner side of the fin. On the inner side of the right pelvic fin only four outgrowths

were found. A single outgrowth was observed on the left side of the anal fin. Six outgrowths were present on the caudal fin of which two were on the right and four on the left side.

Histological: Serial transverse sections (6-8 μ) of tumour, and of the normal fin of the formalin preserved specimen were stained with haematoxylin and eosin, and also according to the Van Gieson's method.

Sections of normal fin (Fig. 2) show the two main layers, the epidermis and the dermis, beneath which lie the fin ray. The epidermis is of stratified nature and has mucous cells, club cells, taste buds, granular cells and pigment cells. The dermis is made up of connective tissue fibers with spindle-shaped or oval nuclei; and pigment cells and blood vessels are also present.

Sections of the tumour (Fig. 3) show that the epidermis over the growth is thinned and pushed out due to the overgrowth of the dermal region. The tumour tissue is composed of interlacing bundles of smooth muscles which take up Van Giesen's stain. The interlacing bundles are separated by thin blood vessels here and there (Fig. 3). Thus the histological picture as well as staining with Van Giesen confirms the growth to be a case of leiomyoma excepting that the nuclear structure is somewhat different. Many nuclei of the tumour cells do not show the typical cigar-shaped structure (Fig. 4), seen in human leiomyomas.

Discussion

Not very many cases of leiomyoma have so far been observed in fishes and of the four recorded cases, three have been reported from the wall of the stomach, by Plehn (1906) in the rainbow trout (*Salmo gairdnerii*), by Pesce (1907) in trout (*Salmo carpio*) and by Thomas (1933) in herring (*Clupea harengus*) respectively. Plehn (1906) also recorded the same type of tumour in the trunk muscles of the shiner (*Alburnus lucidus*). But in fishes none of the previous workers has observed leiomyomas on the skin or with multiple outgrowths. Multiple outgrowths of leiomyoma have, however, been observed in the human uterus as well as in the cutis. But in fishes such outgrowths are rather common in the case of fibroma, and Wago (1922) describes such growths over the body surface, fins and eyes in a goldfish (*Carassius auratus*).

Leiomyoma in human cutis are known to arise from the unstriped

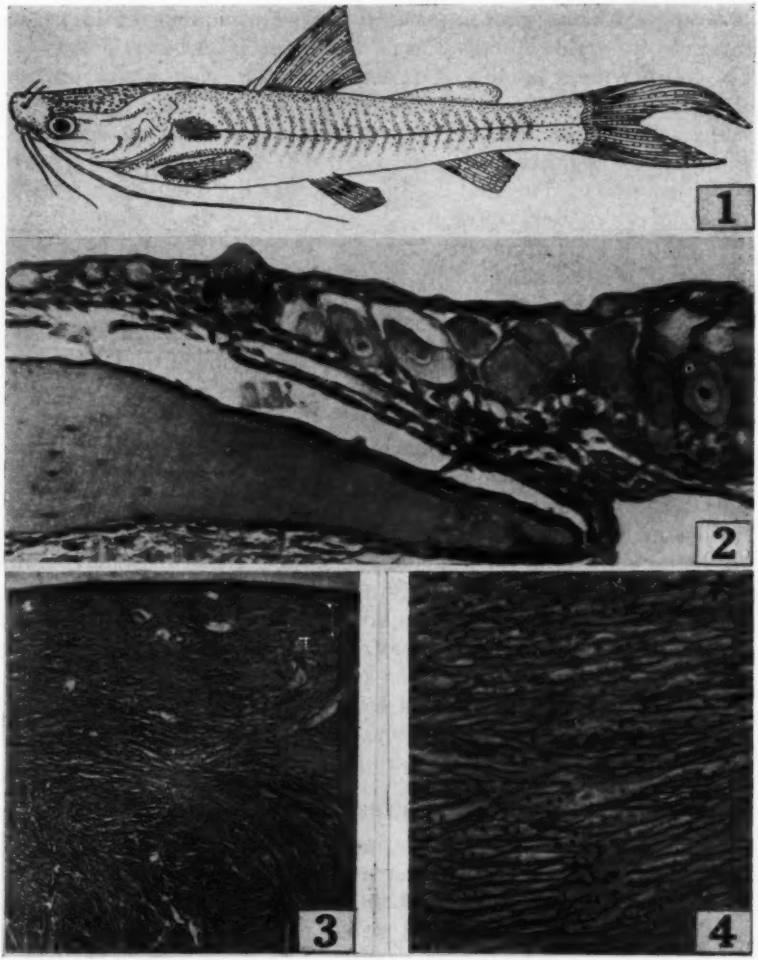

PLATE I

A study of Leiomyoma, a mesenchymal tumour on the fins of an Indian catfish, *Mystus seenghala*.

FIG. 1.—Lateral view of the catfish, *Mystus seenghala*. Black blotches over the fins showing growth sites.

FIG. 2.—Photomicrograph of a section of normal fin of the catfish showing two layers of cells and a part of the fin ray. X 525.

FIG. 3.—Photomicrograph of a section of the catfish tumor under low power. X 135.

FIG. 4.—The same under high power. X 540.

muscle—arrectores pilorum; but this type of muscle could not be observed in sections of the normal fin in our material (Allen, 1954). It may sometimes be difficult to determine the origin of the tumours though one may venture speculation within reasonable limits. Normally some spindle shaped cells are seen just beneath the epidermis; these cells might have the capacity to differentiate into mature cells with various functions and it is possible that this multipotency under the influence of some unknown stimulus may result in their development into either fibroblastic tumours or other tumours of mesenchymal origin including leiomyoma.

There is also another possibility and that is that the latter type of tumour may have arisen from the musculature of the blood vessels. But in that case there would be some difficulty in explaining the occurrences of the multiple growths such as have been found in the present specimen.

The origin of the leiomyoma may be due either to some internal influence such as hormonal imbalance or to some other external agent like virus. We have not made any special study to determine the influence of either of the factors. The nature of the multiple growths of the tumours, however, leads one to speculate that they may be due to some external agency, namely a virus. But human leiomyoma of the uterus which also shows multiple growths, according to some authorities, is due to hormones (oestrogen) acting as an exciting cause. This has, however, not yet been established. Moreover the fish at present under study is obtained from fresh water which is generally not a good source for viral infection. However, for determining the etiology, further investigations are in progress.

Acknowledgments

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Summary

This communication records leiomyoma from *Mystus* (*Oesteobagrus*) *seenghala*. Twenty-three outgrowths of varying sizes are observed in a new site, the fins of the fish. Microscopically, the epidermis of tumour is thinned and pushed out due to the overgrowth of

the dermal region. The tumour tissue consists of interlacing bundles of smooth muscles. The typical cigar-shaped structure of nuclei of human leiomyoma is not observed in this case. Probable histogenesis and etiology of the tumour are discussed.

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THE ROLE OF THE THYROID GLAND IN DIRECT DEVELOPMENT IN THE ANURAN, *ELEUTHERODACTYLUS MARTINICENSIS*

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Although most amphibians, as the name implies, have an aquatic larval stage and a terrestrial adult stage there are several groups in various parts of the world which show striking modifications of the life history by which the aquatic stage is abbreviated or omitted entirely. One such group is the anuran genus Eleutherodactylus of tropical America, the members of which lay their eggs on land and hatch as tiny fully-formed frogs. The intra-oval development has been studied in some detail for at least five species, *E. nubicola* (Lynn, 1942), *E. guentheri* (Lynn & Lutz, 1946a), *E. nasutus* (Lynn & Lutz, 1946b), *E. portoricensis* (Gitlin, 1944), and *E. ricordi planirostris* (Goin, 1947) and incidental observations on isolated embryonic stages have been recorded for quite a number of others. These studies indicate that development in this genus is characterized by the following features. The egg is large (3.0 to 5.0 mm.), yolky, and unpigmented with several membrane-like jelly coatings. Cleavage is holoblastic. The embryo develops a short, highly vascular tail with exaggeratedly enlarged dorsal and ventral fins; the tail begins to decrease in size a few days before hatching and is completely resorbed within a day or two after hatching. Simple external gills appear transiently in some species but are entirely lacking in others. No gill slits or internal gills have been reported for any species and the operculum is reduced to a small vestige (Orton, 1949). Fore and hind limbs appear almost simultaneously. Tadpole-like mouth parts and

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the characteristic lateral-line system of tadpoles are absent. In short, many of the familiar features of an aquatic tadpole are either completely lacking or only slightly represented, the outstanding exception being the tail which is presumably retained as the larval respiratory organ.

In view of the well-known function of the thyroid secretion in the metamorphosis of ordinary anurans the possible role of the thyroid in the direct development of Eleutherodactylus is of special interest. One of us has earlier reported some experiments concerning the effects of thyroid inhibition upon the development of a member of this genus (Lynn, 1948). The present paper embodies the results of a more extensive investigation, involving both thyroid inhibition and thyroxin administration, in which the effects upon the internal organs as well as those upon the external features have been studied.

Materials and Methods

Embryos of *Eleutherodactylus martinicensis* (Tschudi) used for this study were all collected in the immediate vicinity of the University College of the West Indies, Jamaica, B.W.I. Three species of Eleutherodactylus occur in this region but their eggs may be readily distinguished by differences in the structure of the jelly coats, in the number of eggs in a clutch, and in laying sites. *E. martinicensis* is not a native Jamaican species but is well established there as a result of several separate intentional introductions from other parts of the West Indies. In 1952 its eggs were available at the University College throughout most of the year, the supply failing only during a dry period from late November to early January. An account of the natural history, including breeding habits, of the species is being published elsewhere (Lynn, 1955).

For the experiments reported here, 37 clutches of eggs were utilized. The number of eggs in a clutch varied from 10 to 29 and the total number of eggs used was 744. All of the eggs in a single clutch are laid at the same time and are therefore at the same stage of development at any given time. Each clutch of eggs was utilized as a single experimental series, part of the clutch being set aside as controls and part subjected to the appropriate experimental treatment. As has been pointed out in a previous paper (Lynn, 1945) the embryos of Eleutherodactylus, although they ordinarily develop on land, will develop normally in water providing the jelly coatings are re-

moved to permit ready gas diffusion. In the present study all embryos, both controls and experimentals were freed from the coatings and placed in fluid before the start of the experiments. Controls were kept in tap water, experimentals in the desired concentrations of thyroxin or thyroid-inhibiting drug made up in tap water. The medium was changed daily and similarity of environmental conditions was ensured by keeping experimental and control groups side by side on the same laboratory shelf. Feeding was, of course, unnecessary since these animals do not completely use up the yolk of the egg until some days after hatching.

Observations were made daily and embryos were fixed at various intervals for sectioning. Fixation was in a 1:1 mixture of Bouin's fluid and cellosolve. Serial sections were made at 5μ and stained with Mallory's triple stain.

Since all the eggs were collected in the field the exact age was known for none of them. However, three clutches which were in early cleavage to early neural plate when found were set aside to provide a series of normal stages. One animal was preserved from each of these every 24 hours up to hatching. The earliest clutch found was collected at 2:00 P.M. on November 1, 1952 and at that time was judged to be in fourth cleavage. It seems reasonable to assume that these eggs were laid during the morning which would mean that the first four cleavages probably occur in not more than 8 hours. Some eggs from this clutch began hatching 13 days later and all those remaining had hatched by the 14th day. Judging from this, and from the hatching time of several other clutches which were at early stages when found, the normal developmental period of this species is 13 to 14 days at laboratory temperatures. This may be slightly longer than the time required for development in nature since the usual laving sites are in the leaf axils of pinguin plants in open situations where the eggs are exposed to rather high temperatures during the dav.

The external features of the normal series were studied in detail and a set of 13 stages was set up corresponding to 24 hour intervals from fertilization to hatching. Detailed description of the normal stages will not be presented but some of the outstanding features of the early development may be mentioned briefly. The neural plate is well defined at 24 hours (Stage 1). Closure of the neural tube has occurred, both fore and hind limb buds are present, and a short flattened tail, sharply bent to one side has appeared at Stage 2. Stage 3

shows the first appearance of the optic vesicles and the stomodeal invagination as well as growth of limbs and tail. Broadening of the tips of the limbs, foreshadowing the formation of the feet has occurred by Stage 4. Stage 5 is characterized by the division of the fore-limb into proximal and distal segments and the beginning of differentiation of the digits. The further development of digits and limb joints and changes in the head region characterizing the succeeding stages need not be detailed. The egg tooth, a unique feature in these frogs, first appears at Stage 7 and continues to grow until hatching. The tail reaches its maximum size at Stage 10 after which it is gradually reduced though still well-developed at hatching. The egg tooth is lost and the tail shrivels and disappears within one or two days after hatching.

All the eggs used for the experimental work were staged as accurately as possible by reference to this normal series and one may thus compare the results of various experiments even though the precise ages of the eggs used were not known and varied considerably. For most experiments, treatment was begun at Stage 4, 5, or 6, but in several cases the eggs were at Stages 7 to 9 before treatment was instituted and a few clutches were allowed to reach Stage 11 before being used. Experiments were carried on to four to twelve days past the normal hatching time and, since control and experimental animals were always preserved at regular intervals, the progressive effects of the various treatments can be followed on quite closely staged material.

For most of the experiments dealing with thyroid inhibition, phenylthiourea in a concentration of 0.01% was used as the culture medium, a total of 153 embryos being subjected to this treatment. Some 43 embryos were used to test the effects of a lower concentration, 0.005%, of the same drug. Two other thyroid inhibitors, thiourea and thiouracil, were studied using smaller numbers of eggs, 30 in each case. Effects of thyroxin treatment were tested on a total of 81 embryos. The concentrations tried varied from one part of thyroxin in 100 million parts water to one part of thyroxin in one million. Several clutches of eggs were used in experiments designed to study the speed and extent, of recovery from the effects of thyroid inhibition. The embryos were raised, in some cases for 90 hours, in others for 120 hours, in 0.01% phenylthiourea and then removed to tap water, specimens being then preserved at regular intervals. A total of

61 embryos was used in this series. The number of control animals in the various experiments was 185. Embryos used for the establishment of the normal staging numbered 62.

Results

1. *Effects of treatment with thyroid inhibitors*

Preliminary experiments indicated that a 0.01% solution of phenylthiourea produces marked effects upon the development of these frogs without causing any increased mortality or showing any obvious signs of toxicity. This treatment was therefore adopted as the principal method for study of thyroid inhibition, other concentrations and other drugs being tested for comparative purposes only. During the course of the experiments it became clear that the results of administration of 0.01% phenylthiourea vary, not only with the length of treatment, but also with the time at which treatment is instituted. Embryos placed in the solution at Stages 4-6 showed much more striking effects after a given period than did those in which treatment was begun at Stages 7-9 or later. We will therefore consider these two groups separately.

In the experiments started at Stage 4 the first grossly visible differences between experimental and control embryos were noted on the third day after the initiation of treatment. At this time the experimental animals showed a noticeable retardation in limb differentiation, the digits being represented by small lobes, while those of the controls were tapering and showed well-defined joints. By the fifth day of treatment the contrast between the limbs of experimental and control animals was even more striking, the total length of the hind limbs of the controls now being approximately twice that of the experimental limbs. However, it was also clear by this time that the treated animals were retarded in general development; the body length was less than that of the controls and there was a correspondingly greater amount of unused yolk present. The egg tooth had differentiated in both, but was quite small in the experimental animals. Examination of specimens at successively later periods showed increasing contrast in growth of limbs and body and when the controls had reached Stage 13 (hatching stage) their body length averaged 5.5 mm. while that of the experimental animals was 4.0 mm. The average length of the hind limb was 8.0 in the controls, 4.5 mm. in the experimentals. As has been mentioned, the tail is normally

lost shortly after hatching and this occurred in the controls by the tenth day of treatment. In the treated animals the tail was still at its maximum size, 3.0 mm. long and 2.5 mm. wide, at this time (compare Figures 11 and 12). No reduction in tail size occurred so long as treatment was continued. It is noteworthy that, although growth and differentiation were definitely retarded in these experiments, they did occur and complete differentiation of digits and limb joints was ultimately accomplished even though at a considerably later time than in the controls. The only external features which seemed to be completely inhibited were tail resorption and, possibly, loss of the egg tooth. Tail resorption failed to occur even after the experimental animals had reached developmental stages corresponding to post-hatching stages. The egg tooth was similarly retained in most embryos but in a few it dropped off late in the experimental period. It is therefore possible that this latter feature is one which is exceptionally retarded rather than completely inhibited.

Experimental series in which treatment was started at Stage 5 or 6 showed no detectible effects until 5 days after the beginning of treatment at which time the limbs of control and experimental animals differed in length and digital differentiation. Later, a contrast in limb development, body length, and yolk absorption became increasingly apparent and by the time the controls reached Stage 13 the differences between them and the experimental embryos were essentially the same as those described for the series begun at Stage 4.

The results when treatment was initiated at Stages 7-9 were somewhat different. In these cases, no marked retardation of limb or body growth occurred by the hatching stage; controls and treated animals were quite similar in appearance at this time. Later, however, by two days past the hatching stage, complete tail resorption and loss of the egg tooth had occurred in all controls while the experimentals retained the egg tooth and had tails of maximum size. This was still the case in embryos carried two weeks beyond the normal hatching stage.

Study of the external characters of experimental and control animals thus indicated that treatment with 0.01% phenylthiourea, initiated at an early stage, causes a marked general retardation of growth and differentiation and prolonged retention of the larval tail and egg tooth. When the treatment is begun late no externally detectible effect on growth and differentiation is produced but the egg tooth and tail are affected.

Selected control and experimental animals representing various times of initiation of phenylthiourea administration and various periods of treatment were sectioned for study of the internal organs. Since the primary purpose of the investigation was to ascertain the role of the thyroid in the direct development of these frogs by studying the results of inhibiting thyroid function, attention was given first to the histology of the thyroid as a check on the effectiveness of the treatment. It was at once apparent that the thyroids of all experimental animals, regardless of whether they were treated at early or late stages, showed clear-cut effects which were always distinguishable by the third day of treatment and steadily increased to a maximum by the ninth day. Further treatment did not seem to cause any more pronounced effects, the thyroids remaining in a state of marked hypertrophy. The effects are illustrated in a series of photographs (Figures 1 to 6) comparing the thyroids of experimental embryos with those of the corresponding controls. At 4 days the thyroids of treated animals show an increase in the height of the follicular epithelium, the presence of a moderate number of chromophobe droplets at the peripheries of the colloid masses, and a slight hyperemia as compared with the control glands. There is no hypertrophy of the gland as a whole, however, and there is no indication of a significant increase in mitotic rate in the epithelial cells. By the fifth day (Figures 1 and 2) hypertrophy has begun, the increase in epithelial height is still more marked, and much of the colloid has disappeared from the follicles. At eight days (Figures 3 and 4) further hypertrophy has occurred and the follicle cells are highly vacuolated and on the ninth day (Figures 5 and 6) the contrast between the thyroids of control and experimental animals is most striking. The controls now show rather inactive thyroids with flattened epithelium and much stored colloid while the experimental thyroids have a high, vacuolated, much folded epithelium and most follicles contain no colloid at all. Volume determinations based on measurement of all sections in 20 thyroids of animals treated for 6 to 12 days give an average of 0.221 cu. mm., while similar determinations for the corresponding controls give an average of 0.104 cu. mm. It seems quite evident that treatment with 0.01% phenylthiourea did bring about all the histological changes in the thyroid which are now well-recognized as indications of quite effective inhibition of normal thyroid function.

We may now turn to consideration of the effect of the thyroid in-

hibition upon the development of internal organs. The serial sections were utilized to study the embryology of digestive, vascular, and urogenital systems and the sequence of ossification of the skull bones in experimental and control embryos, special attention being given to structures which, in ordinary anurans, show marked changes at the time of metamorphosis.

In the digestive tract the following features were studied in detail; development of the pharyngeal derivatives and lungs, histological differentiation of the walls of stomach and intestine, origin and histo-differentiation of liver, gall bladder, and urinary bladder, and rate of yolk absorption. In all of these features, embryos treated with 0.01% phenylthiourea from Stage 4 show a retardation as compared with controls. In the differentiation of the pharyngeal derivatives this is already apparent on the third day of treatment but for the other features, differences are not demonstrable with certainty until the fourth day. After this time the contrast between exprimentals and controls is increasingly evident. Nevertheless, the digestive tracts of the experimental embryos did differentiate steadily and ultimately attained all the characteristic differentiations of the Stage 13 controls. However, even at this time, the unused yolk in the experimental animals far exceeds that normally present at Stage 13. It is worthy of special note that Hassall's corpuscles appear in the thymus of experimental animals, though only about 6 days later than they appear in controls.

In the vascular system the same situation is observed; the development of the aortic arches, pulmonary vessels and posterior vena cava all being retarded but proceeding normally in the treated embryos.

The sequence of ossification of skull bones was studied because most of the bones are formed in ordinary anurans as a definite part of the metamorphic pattern (Erdmann, 1933). Of most interest are the angular, dentary, maxilla and squamosal which, in Rana, ossify just at metamorphic climax and might therefore be presumed to depend upon thyroid functioning. Again, however, study of the present material shows that these bones ossify normally, though at a later age, in the embryos receiving treatment.

Thus in all these systems we have evidence of a striking general retardation of normal development which results however, at any par-

ticular stage, in a harmonious combination of features precisely corresponding to that found in the controls at an earlier age.

The urogenital system provides the only clear-cut instance of a disharmonious retention at late stages of an internal organ characteristic of early development. This is the pronephros. In control embryos the pronephric tubules first appear at Stage 4, the pronephric ducts passing caudally to enter the cloaca. The tubules increase in size during the succeeding stages and the pronephros reaches its maximum size at Stages 7 or 8. At this time it measures approximately 350 micra in length. After this, as the mesonephros comes into function, the pronephros begins to show degenerative changes in its cells and the tubules become occluded. Such changes are seen in Stages 9 and 10, at first involving only a few tubules but gradually extending to the whole organ. No definite decrease in size of the pronephros occurs until Stage 11 when the average length has been reduced to about 200 micra. By Stage 13 the pronephros is clearly non-functional but can easily be distinguished as a small mass of tissue about 90 micra long. After this it is rapidly resorbed and can be distinguished only with difficulty in post-hatching stages. In embryos raised in 0.01% phenylthiourea from Stage 4 the history of the pronephros corresponds with that for the controls during the growth phases. However, the experimental animals never undergo any degeneration or decrease in size of the pronephros so long as treatment is continued. As a matter of fact the pronephros continues to grow somewhat reaching lengths of 400 to 435 micra, larger than has been found in any control animal. This is illustrated in Figures 13 to 16 which show the pronephroi of experimental and control embryos after 6 days and 11 days of treatment. The pronephric tubules of the treated animals are large and functional in both cases. The control pronephros for the 6-day experiment shows degenerative processes in progress, that for the 11-day experiment shows maximal resorption.

The results of the experiments in which 0.005% phenylthiourea was used may be dismissed briefly, for they correspond precisely with those produced by 0.01% phenylthiourea. Judging from the features visible externally it was suspected, even during the course of the experiments, that the lower concentration was just as effective as the higher and this was confirmed by later study of the thyroid histology and organ differentiation. The effects observed and the times required for their appearance differ in no significant way from those described

above. This is of interest, however, for it seems a good indication that the 0.01% phenylthiourea treatment must certainly have produced the maximum thyroid inhibition obtainable with this drug.

We may now consider the results of treatment with two other thyroid inhibitors, thiourea and thiouracil. At a concentration of 0.01% neither of these drugs proved as effective as 0.01% phenylthiourea. Embryos receiving treatment from Stage 4 showed some retardation of limb growth but it was much less striking than that caused by phenylthiourea-treatment. Nevertheless, retention of tail and egg-tooth did occur both in embryos treated early and in those treated late. The thyroids of animals raised in 0.01% thiourea or 0.01% thiouracil also evidenced only slight effects. Slight increase in epithelial height occurred and some vacuolization of the cells was noted. There was no hypertrophy of the thyroid however and no marked colloid release was evidenced at any stage. Figure 7 illustrates the effect of 8 days treatment with 0.01% thiourea and Figure 8 shows the thyroid of an embryo treated for 11 days with 0.01% thiouracil. Study of the internal organs is also indicative of but slight effect, some retardation in growth and differentiation being observed but only after relatively long treatment. The onset of degeneration of the pronephros was delayed in these embryos until Stage 13 or later but by the fourth day after the controls had reached hatching stages, the pronephroi of the experimental animals had completely disappeared. Unfortunately, the only other concentration of thiouracil used was a weaker one, 0.005%, and, as would be expected, this proved even less effective. Animals raised in this solution kept pace with the controls and lost both tail and egg-tooth at the usual time. For thiourea a stronger concentration, 0.05%, was tested with one series of eggs. These were at Stage 8 when treatment was started and the experiment was continued for 12 days. The effects on external characteristics and on internal organs, including the thyroid, were quite comparable to those seen in embryos treated with 0.01% phenylthiourea at corresponding stages. One may conclude that 0.05% thiourea probably causes maximal thyroid inhibition while concentrations of 0.01% or lower, of either thiourea or thiouracil, do not.

It is necessary to note briefly that there was one major effect observed in certain of the experimental treatments which has not been mentioned previously because it is clearly not a result of thyroid inhibition. This is the depigmentation of the skin and eyes which occurs

in embryos treated with phenylthiourea or thiouracil but not in those given thiourea. Even the lowest concentrations of phenylthiourea and thiouracil were fully effective in producing this blanching which was grossly discernible within 48 to 72 hours after the beginning of treatment. The effect is apparently exerted directly upon the formation of melanin, probably through inhibition of tyrosinase activity. A study of this effect in the embryos of *E. martinicensis* has been published elsewhere (Millott & Lynn, 1954).

2. *Recovery from treatment with thyroid inhibitors*

Previous investigations concerning the thyroid-inhibiting effects of thiourea and its derivatives in various vertebrates have shown that the effects are readily reversible, thyroid activity returning to normal within a short time after cessation of treatment. To test this in the present material several clutches of eggs at Stages 4 and 5 were first divided, as usual, into control and experimental groups, the experimental animals being placed in 0.01% phenylthiourea and allowed to remain, in some experiments for 90 hours, in others for 120 hours. Some embryos were then returned to tap water while treatment was continued on others. Observations and preservation of specimens for sectioning were carried out at frequent intervals up to times when the untreated controls had reached post-hatching stages.

During the course of these experiments, comparison of animals still receiving treatment with those whose treatment had been discontinued showed a clear-cut difference after only 36 hours. This difference, however, concerned the pigmentation. Cessation of treatment is followed by prompt return of melanin and, as a matter of fact, it was later found upon cytological examination of the melanophores, that the reappearance of melanin in the cells of the skin and retina can be demonstrated in embryos which had been returned to tap water for only 8 hours (Millott and Lynn, 1954). As noted above, the effect of phenylthiourea upon melanin formation does not appear to be intermediated through the thyroid and is therefore not pertinent to the present study. Aside from the pigmentary difference, the first signs of recovery were seen between 96 and 120 hours after treatment was stopped. At this time the differentiation of digits, length of limbs, and general body size of the recovery series were clearly intermediate between those of the untreated control series and those of the series subjected to uninterrupted treatment. The recovery became

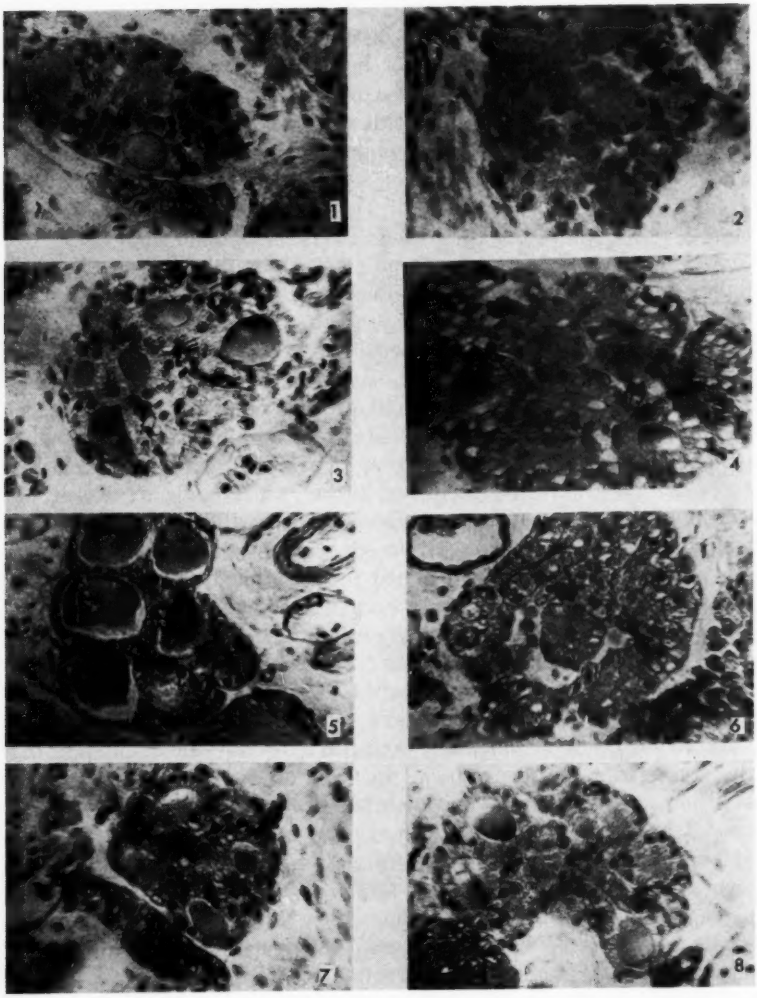
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PLATE I

1. Central section of the thyroid gland of a control embryo of Series H placed in tap water at Stage 4 and fixed 5 days later. X 430.
2. Central section of the thyroid gland of an embryo of Series H placed in 0.01% phenylthiourea solution at Stage 4 and fixed 5 days later. X 430.
3. Central section of the thyroid gland of a control embryo of Series H placed in tap water at Stage 4 and fixed 8 days later. X 430.
4. Central section of the thyroid gland of an embryo of Series H placed in 0.01% phenylthiourea solution at Stage 4 and fixed 8 days later. X 430.
5. Central section of the thyroid gland of a control embryo of Series E placed in tap water at Stage 6 and fixed 9 days later. X 430.
6. Central section of the thyroid gland of an embryo of Series E placed in 0.01% phenylthiourea solution at Stage 6 and fixed 9 days later. X 430.
7. Central section of the thyroid gland of an embryo of Series R placed in 0.01% thiourea solution at Stage 6 and fixed 8 days later. X 430.
8. Central section of the thyroid gland of an embryo of Series R placed in 0.01% thiouracil solution at Stage 6 and fixed 11 days later. X 430.

more obvious with advancing time and the recovery series gradually approached the condition of the controls. In embryos kept in phenylthiourea solution for only 90 hours the recovery was sufficiently advanced that the animals lost the tails and egg-tooth at the same time as did the controls. The embryos treated for 120 hours before return to tap water, recovered somewhat more slowly but most lost the tail within two days after this had occurred in the controls. The embryos which were not removed from the solution, of course retained the tails at maximum size.

Sections were prepared of embryos taken at 12-hour intervals throughout the first 48 hours of recovery and at 24-hour intervals thereafter up to 144 hours. Appropriate untreated control animals and animals subjected to continued treatment, were also sectioned. Examination of the thyroid glands of these specimens shows the first clear indications of return to normal functioning at 72 hours. This is true in both the 90-hour and the 120-hour series. After 72 hours' recovery the follicular epithelium has decreased in height and a considerable amount of colloid is present in all follicles. However the gland is still hypertrophied and hyperemic. At 96 and 120 hours the hypertrophy has disappeared and the thyroids of the recovery series are quite indistinguishable from those of untreated controls.

Study of the development of internal organs confirms the general indications provided by the observations on external features. By three days after cessation of treatment the digestive tract and vascular system in the recovery series are clearly advanced in development as compared with the condition seen in embryos still receiving treatment. In these features the animals are, however, still somewhat behind the untreated controls even up to two days beyond the normal hatching time. In view of the effects on the pronephros reported in the preceding section, special attention was given to this organ. In embryos treated with phenylthiourea for 90 hours the pronephros is large and functional in both experimentals and controls (the controls then being at Stage 8). In animals which had been removed from the phenylthiourea solution for 48 hours the pronephros is still large and well-developed while the controls (now at Stage 10) show the onset of pronephric degeneration, some tubules being occluded and many cells showing highly vacuolized cytoplasm and pycnotic nuclei. At 72 hours after cessation of treatment the pronephros is still unchanged while that of the control is reduced in size and obviously non-functional.

The first signs of degeneration of the pronephros in the recovery series are seen at 96 hours by which time the untreated controls have lost their structure completely. Once degeneration has begun in the experimental animals it progresses even more rapidly than normally and by 120 hours the pronephros has disappeared. Embryos treated for 120 hours before being placed in tap water, somewhat surprisingly, show an even more rapid onset of pronephric degeneration than the 90-hour series. In the 120-hour series degeneration began by 48 hours after cessation of treatment, was well advanced by 72 hours, and completed by 96 hours. These embryos were, of course, at a more advanced stage when treatment was stopped than were the 90-hour treated animals. It appears that, with respect to degeneration of the pronephros the speed of recovery is correlated with the developmental stage.

3. *Effects of thyroxin administration*

In the first experiments dealing with thyroxin administration three different concentrations were tested. These were: one part thyroxin in one hundred million parts of water, one part in fifty million, and one part in ten million. Since the two lower concentrations did not seem to be giving appreciable effects, later series were run with concentrations of one part in five million and one part in one million. Most of these experiments were begun at early stages (Stages 5 or 6). During the progress of the experiments the results were unexpected. No effect on growth or differentiation of the limbs or body were observed and the egg tooth showed no precocity in differentiation or loss. However, the tail was definitely affected. Within four days after the beginning of treatment with thyroxin solutions of 1:10 million or stronger, the tail became crinkled and thickened at the edges with signs of rupture of capillaries near the surface. It then began to shrivel rapidly being reduced to half size by the fifth day and to a small flap by the seventh day. At this time the tails of control embryos were just beginning their pre-hatching reduction.

The thyroid picture agrees with the above observations in that the thyroids of embryos treated with 1:100 million and 1:50 million thyroxin solutions do not differ from those of the controls even after as long as 15 days treatment. On the other hand, the thyroids of animals raised in 1:10 million, 1:5 million, or 1:1 million solutions show decreased activity as evidenced by a flattened epithelium, in-

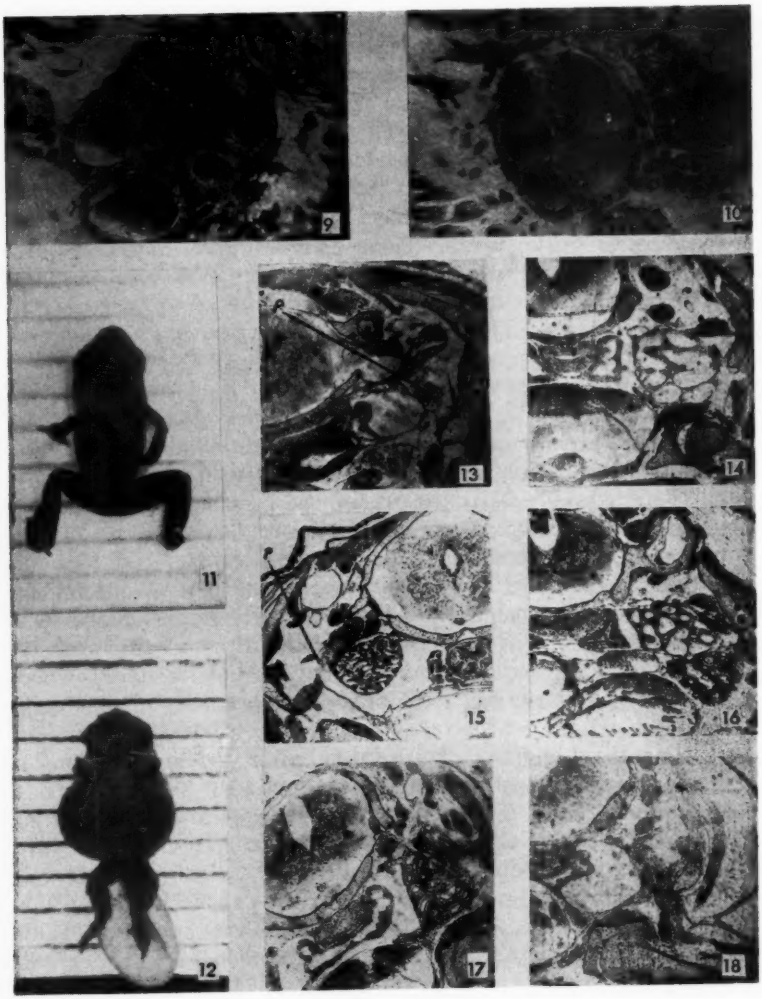
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PLATE II

9. Central section of the thyroid gland of a control embryo of Series BB placed in tap water at Stage 6 and fixed 9 days later. X 430.
10. Central section of the thyroid gland of an embryo of Series BB placed in a solution of one part thyroxin to ten million parts water at Stage 6 and fixed 9 days later. X 430.
11. Ventral view of a control embryo of Series H placed in tap water at Stage 4 and preserved 11 days later. Millimeter rule in background. X 6.5.
12. Ventral view of an embryo of Series H placed in 0.01% phenylthiourea solution at Stage 4 and preserved 11 days later. Millimeter rule in background. X 6.5.
13. Section through the pronephros of a control embryo of Series G placed in tap water at Stage 6 and fixed 6 days later. P, pronephros. X 60.
14. Section through the pronephros of an embryo of Series G placed in 0.01% phenylthiourea solution at Stage 6 and fixed 6 days later. X 60.
15. Section through the pronephros of a control embryo of Series G placed in tap water at Stage 6 and fixed 12 days later. X 60.
16. Section through the pronephros of an embryo of Series G placed in 0.01% phenylthiourea solution at Stage 6 and fixed 12 days later. X 60.
17. Section through the pronephros of a control embryo of Series CC placed in tap water at Stage 5 and fixed 5 days later. X 60.
18. Section through the pronephros of an embryo of Series CC placed in a solution of one part thyroxin to five million parts water at Stage 5 and fixed 5 days later. X 60.

crease in stored colloid, and absence of chromophobe colloid. The contrast between the glands of control and experimental animals can in no case be made out with certainty before the seventh day of treatment but after this the difference is well-marked (Compare Figures 9 and 10).

Study of the differentiation of the internal organs, as might perhaps have been expected on the basis of the external features, shows, in general, no significant difference between experimental and control embryos. Again, however, the degeneration of the pronephros seems to be definitely under thyroid control. In embryos treated with the three higher concentrations of thyroxin, the pronephros begins to show signs of involution after 4 days of treatment (at Stage 9) and by Stage 10 (5 days of treatment) has already been reduced to a point which is not reached in the controls until Stage 13 (Figures 17 and 18).

Discussion

The normal embryology of the frog used in this investigation, *Eleutherodactylus martinicensis* (Tschudi), has not previously been studied despite the fact that this was one of the first members of the genus for which direct development was recorded (Bavay, 1873). Bavay's observations, made on eggs collected in Guadeloupe, were confined to external features. Some further details on the timing and sequence of external changes have been provided much more recently by Iris Bayley (1950) in connection with her investigation of ciliary activity in this species. On the basis of the study of the normal stages and the untreated controls in the present work it is clear that the general features of the embryogeny of *E. martinicensis* agree closely with those of *E. nubicola* (Lynn, 1942). The only really significant difference lies in the fact that *E. martinicensis* shows the transient appearance of a single pair of external gills. These can be seen in living embryos of Stages 5 and 6 as small bright red projections from the neck region. They disappear, however, by Stage 7. In the sectioned material they prove to be simple loops from the carotid arch. These structures were noted in this species by Bavay (1873) and they also occur in *E. portoricensis* (Gitlin, 1944). *E. nubicola* has a rather long intraoval period (26 days) but this is probably related to the fact that it is a mountain form living in regions where the average temperatures are fairly low. Gitlin (1944) reports a developmental period of 17.5 to 19.5 days for *E. portoricensis* and Goin

(1947) finds an average period of 15.6 days for *E. ricordii planirostris*. As has been noted, the eggs used for the present study were raised under laboratory conditions and the developmental period of 13 to 14 days may be somewhat longer than that of eggs in the normal laying sites. As a matter of fact both Bavay (1873) and Bayley (1950) report that hatching occurs on the 10th or 11th day in this species.

The use of thiourea and its derivatives as a method of inhibiting the production or release of the thyroid hormone is now well known. Although the precise mechanism of action is still uncertain (Astwood, 1949) there is no doubt that these drugs provide an effective means of "chemical thyroidectomy" which is of great aid as an investigative tool in forms in which surgical thyroidectomy presents special difficulties. In the present work three different thyroid inhibitors were used in various concentrations and their effectiveness was judged by later study of the histology of the thyroids of experimental and control animals. Phenylthiourea at concentrations of 0.005% and 0.01% produced rapid changes in the thyroid (first detectible within 4 days after the beginning of treatment) indicating that maximal thyroid inhibition was effected. Thiourea and thiouracil at a concentration of 0.01% caused changes which were not maximal, but 0.05% thiourea proved just as effective as the phenylthiourea treatments. It seems justifiable to conclude that embryos raised in 0.005% or 0.01% phenylthiourea or in 0.05% thiourea were developing without any normal thyroid secretion.

In the frogs of the genus Eleutherodactylus, which attain many of the adult differentiations while still in the egg and omit many characteristic anuran larval features entirely, the role of the thyroid during intraoval development is of special interest. In ordinary anurans many of the adult differentiations are known to be directly dependent upon thyroid functioning, and they fail to occur when the thyroid is removed or inhibited (see review of Lynn & Wachowski, 1951). The question arises whether the direct development of Eleutherodactylus is connected with precocious thyroid activity or whether on the other hand, the differentiations which usually depend upon the thyroid are somehow carried out independently in this frog. In an earlier study of the effects of thyroid inhibition upon the external features of development in Eleutherodactylus (Lynn, 1948) it was concluded that two features, differentiation of the hind limbs and re-

sorption of the tail, give evidence of being under thyroid control. The present, more detailed, experiments necessitate some modification of this concept and also provide information on internal differentiations.

It now seems clear that the limb differentiation cannot truly be considered to be dependent upon the thyroid, at least not in the sense that hind-limb growth depends upon thyroid activity in ordinary anurans. In Rana, for example, treatment of the tadpole with effective concentrations of thyroid inhibitors results in complete cessation of hind limb growth and differentiation (Gordon, Goldsmith and Charipper, 1943; Hughes and Astwood, 1944; Lynn and Sister Alfred de Marie, 1946). In Eleutherodactylus, on the other hand, although the rate of growth and differentiation of the limbs is quickly and markedly decreased by the treatment, the limbs do ultimately differentiate fully and do continue to grow. The development of internal organs shows the same general retardation and it seems obvious that the limb differentiation is simply one of many processes which are retarded but not truly inhibited in these experiments. There are two exceptions, however. One is the pronephros which remains as a large functional organ in the treated animals even when, in other features, the body has differentiated well beyond the stage when degeneration of the pronephros should have occurred. This same statement applies to the larval tail which persists at maximum size in the experimental animals so long as treatment is continued.

One may, with justice, question whether the effects of the drugs upon general growth and differentiation are to be ascribed to the thyroid inhibition at all. It is quite conceivable that they are due to direct toxic effects. Although it is clear from the histological picture presented by the thyroid, that effective thyroid inhibition did occur in these experiments, we know relatively little of the side effects of these drugs, particularly in lower vertebrates, and a retardation of developmental processes is a commonly observed effect of treatment with various harmful agents. Resolution of this question must await development of methods for performing surgical thyroidectomy in these animals which would permit study of the absence of thyroid hormone without the complicating effects of drug administration. It does not seem likely that toxicity could account for the effects on pronephros and tail, however. We feel that the evidence indicates that the normal changes in these two structures are definitely dependent upon thyroid function. It is noteworthy that both are processes

involving degeneration and resorption rather than differentiation and growth. It is difficult to imagine that toxic effects could result in indefinite retention of structures normally resorbed, as seems to be the case here. Whether the loss of the egg-tooth belongs in this same category is uncertain since the experimental results were somewhat variable as regards this feature.

The fact that the histological changes which occur in the thyroid after administration of thyroid-inhibiting drugs are quite readily reversible when treatment is stopped, has been demonstrated in a number of studies. In the present work, the return of the thyroid to the histological appearance characteristic of normal functioning occurred within 72 to 96 hours after cessation of treatment. Recovery from the effects on growth and differentiation also occurred within a relatively short period and resorption of pronephros and tail, though somewhat delayed as compared with untreated controls, was carried out normally. Animals which had reached a more advanced stage when treatment was stopped showed a more rapid degeneration of the pronephros than did those of earlier stages. It appears that an older pronephros, retained in the body well past the stage when it should have degenerated completely, has a greater tendency to degenerate when treatment is discontinued than does a younger one which has been retained into stages when degeneration would normally have been in progress but not complete. It may be that this is connected with specific age changes in the tissues.

Thyroids of embryos treated with thyroxin showed the same indications of decreased activity that have been demonstrated for both homeotherms and poikilotherms (Eggert, 1935). In the Eleutherodactylus embryo, however, unusually high concentrations were required to obtain these effects in the necessarily short periods during which the experiments were run. The thyroxin experiments serve to strengthen the conclusions previously stated for, while the rate of growth and differentiation of most external structures and internal organs was not in any way affected, the pronephros and tail showed prompt and specific response, degenerating well before the normal time.

We may conclude that most of the differentiations which occur at the time of metamorphosis in ordinary anurans and are known to be under thyroid control have, in the evolution of direct development in the genus Eleutherodactylus, been emancipated from such control. They can occur under conditions of maximal thyroid inhibition and

they are not stimulated by administration of quite high concentrations of thyroxin. The features which are definitely affected by thyroid inhibition or thyroxin treatment, tail resorption, pronephros degeneration, and possibly the loss of the egg-tooth, are all events characteristic of late embryonic stages and involving retrogressive processes.

Summary

1. The normal embryology of *Eleutherodactylus martinicensis* has been studied and a series of normal stages has been established to serve as a basis for comparison with experimentally treated embryos.

2. Treatment of embryos with 0.01% or 0.005% phenylthiourea or 0.05% thiourea results in maximal inhibition of thyroid activity, as judged by the histology of the thyroid gland.

3. Animals receiving such treatment from the fourth or fifth day of development are markedly retarded in the growth and differentiation of body, limbs, and organ systems. Nevertheless both growth and differentiation are completed and the treated embryos ultimately attain the size and structure of newly hatched controls, though at a much later time. Three features, degeneration of the pronephros, resorption of the tail, and loss of the egg-tooth, do not occur in the treated embryos so long as the experiments are continued. These three features thus appear to be the only ones definitely dependent upon thyroid function.

4. Embryos removed from the thyroid-inhibiting drugs after 90 or 120 hours' treatment show a quick recovery in the histology of the thyroid, in the rate of growth and differentiation, and in the loss of the three structures mentioned above.

5. Administration of thyroxin, at concentrations of 1 part in 10 million or higher, produces characteristic changes in the thyroid indicative of decreased secretory activity. This treatment has no effect upon the rate of growth and differentiation but does cause precocious degeneration of the pronephros and resorption of the tail.

6. It is suggested that, in view of the fact that thyroxin treatment does not affect the growth rate, the retardation observed in the experiments with thyroid inhibitors may well be attributable to direct toxic effects of the drugs rather than to the thyroid inhibition. According to this hypothesis, the only features specifically affected by the thyroid inhibition are those which also respond to thyroxin treatment; i.e. pronephros degeneration, tail resorption, and possibly loss of the egg-tooth.

7. Direct development in Eleutherodactylus seems to involve a considerable modification of the mechanism by which many of the late developmental features are attained in ordinary anurans. The usual events of "metamorphic climax" are carried out without dependence upon the pituitary-thyroid complex and only a few degenerative changes seem to retain complete dependence upon thyroid functioning.

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GROWTH CHARACTERISTICS OF AVENA SEEDLINGS GROWN FROM GAMMA IRRADIATED SEEDS[1]

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INTRODUCTION

Much of the experimental work on the effects of gamma radiation on the seeds of higher plants has been concerned with the process of germination (Petit and Ancelin, 1913; Stoklasa, 1913; Agulhon and Robert, 1915; Redfield and Bright, 1922). Responses of intact seedlings to ionizing radiation have been reported less frequently. Tanada (1952) observed an inhibitory effect of gamma rays on the growth of one-day old barley roots, which was increased by oxygen concentrations up to 5 per cent. This paper presents a study of some of the growth characteristics of the coleoptiles and roots of Avena seedlings sprouted from seeds which previously had been exposed to various dosages of gamma radiation from radioactive cobalt.

MATERIALS AND METHODS

Avena sativa seeds of the Victory Strain (U. S. Department of Agriculture, C. I. 2020)[3] were used. The seeds were husked and divided into two aliquots. One aliquot was placed in a relative humidity of 87 per cent until the seeds reached constant weight. Preliminary observations had indicated that this treatment resulted in more uniform seedlings with maximum coleoptile length. The second aliquot of air dried seeds was kept in room atmosphere. All seeds were transferred to a lucite and lead irradiation chamber which was constructed to keep the seeds in a single layer. The irradiation chamber surrounded an aluminum-encapsulated source of $_{27}Co^{60}$ (1.16;

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1.31 mev gamma). The initial dose rate of the 95.35 mc source approximated 317 Roentgens/hour at a mean distance of 2 cm. Calculation of the exposure time for each aliquot of seeds was based on a half-life of 5.27 years.

Subsequent to irradiation the seeds were soaked in Shive's solution for 4 hours and then plated in Petri dishes on filter paper saturated with Shive's solution. While the seeds were in the Petri dishes they were exposed to weak red light to inhibit the development of the first internode. Forty-eight hours after the initiation of soaking the seedlings were transplanted to individual glass holders in aquaria also containing Shive's solution. The seedlings were then allowed to grow in the dark to the age of 96 hours. Measurements and other experiments were then initiated.

Experimental Results

Coleoptile growth

Figure 1 shows the effects of gamma irradiation of seeds on the length of subsequently grown coleoptiles. Curve I was obtained at 23 (± 1) degrees C from seeds equilibrated at 87 per cent relative hu-

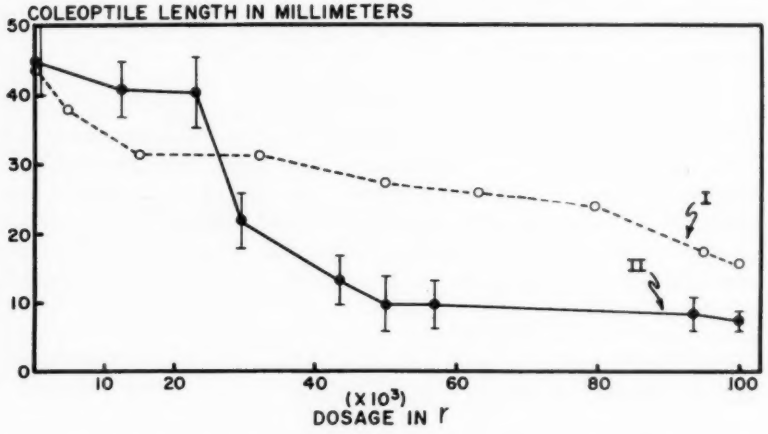

FIGURE 1

The effect of gamma irradiation of *Avena* seeds on coleoptile length. Each point represents an average of 40 or more measurements.

Curve I is from seeds that were equilibrated at 87 per cent relative humidity before being irradiated.

Curve II is from air dried seeds, in equilibrium with room air.

midity which results in a 10 per cent increase in weight. The standard deviations for the points in this curve are similar in magnitude to those indicated by the vertical lines in Curve II. The measurements for Curve II were obtained from seeds that were not humidified (air dried) prior to irradiation. Although the higher doses caused a marked inhibition of coleoptile growth, it is significant to note that doses up to 100,000 r had no effect on germination numbers. Comparison of Curves I and II indicates that humidification of seeds makes them more sensitive to low dosage irradiation. Conversely, above 30,000 r, humidification has a protective effect.

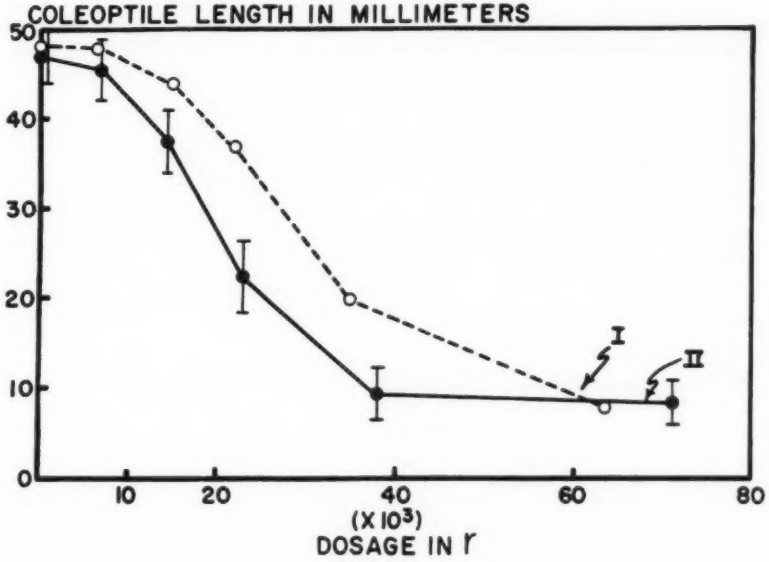

FIGURE 2

The effect of gamma irradiation of *Avena* seeds on coleoptile length. Each point represents an average of 40 or more measurements.
Curve I is from air dried seeds irradiated at 13 (± 1.0)° C.
Curve II is from air dried seeds irradiated at 1.2 (± 0.8)° C.

Figure 2 shows the effects of irradiating air dried seeds at 1.2 (± 0.8) and 13 (± 1.0)° C on coleoptile growth. Curve II corresponds to the lower temperature. The inhibitory effects of gamma radiation were influenced to a limited extent by temperature treatment.

A comparison of the curves in Figure 2 with Curve II in Figure 1 shows a slight increase in sensitivity with decreasing temperature, but the general shape of the curves is the same. That this effect was not due to exposure to cold alone, was shown by appropriate controls. However, the maximum inhibition of coleoptile growth was attained between 40,000 and 60,000 r in all experiments in which air dried seeds were used.

Root growth

Figures 3 and 4 show the effects of gamma irradiation of seeds on the growth of the primary roots. Curve I in Figure 3 is from

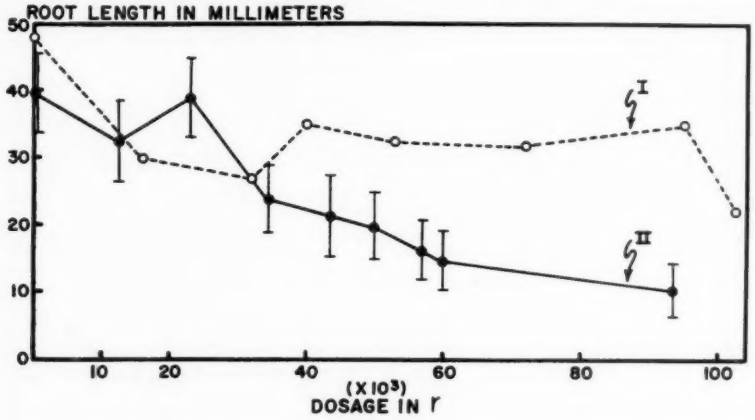

FIGURE 3

The effect of gamma irradiation of *Avena* seeds on primary root length. Each point represents an average of 40 or more measurements.

Curve I is from seeds that equilibrated at 87 per cent relative humidity before irradiation.

Curve II is from air dried seeds, in equilibrium with room air.

seeds that were humidified prior to irradiation, while Curve II is from air dried seeds. As previously shown for coleoptiles, humidification decreases the inhibitory effect of radiation when doses above 40,000 r are applied. Below 40,000 r some differences were apparent. Roots from non-irradiated, humidified seeds grow longer than the roots from dry seeds. Also, Curve II shows 13,000 r to be more inhibitory to root growth than 23,000 r. The difference between these

points is statistically significant (P = less than 0.05), but the cause of this difference is not understood at the present time.

Growth rates of roots from air dried seeds that were irradiated at 1.2° C resemble those of roots from seeds irradiated at 23° C. Compare Curve II of Figure 4 to Curve II of Figure 3 for this observation. It should be noted that Curve II of Figure 4, although it is somewhat compressed, shows the same sequence of events indicated by

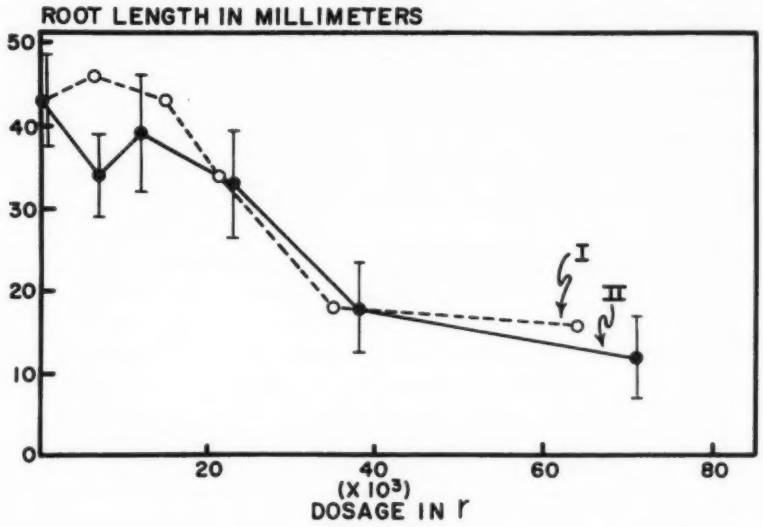

FIGURE 4

The effect of gamma irradiation of *Avena* seeds on primary root length. Each point represents an average of 40 or more measurements.
Curve I is from air dried seeds irradiated at 13 (± 1.0)° C.
Curve II is from air dried seeds irradiated at 1.2 (± 0.8)° C.

the corresponding curve in the previous Figure. When irradiation is applied to air dried seeds at 13° C, the inhibitory effect on root growth was induced only by doses above 15,000 r.

One other observation can be made at this point. Complete inhibition of the development of the second pair of adventitious roots was noted on some of the seedlings grown from humidified seeds which had been exposed to 25,000 r. Smaller doses sometimes resulted in decreased length of these roots, and in a few instances only one mem-

ber of the pair was completely inhibited. The frequency of complete inhibition of both of the second adventitious roots increased with the dose up to about 50,000 r. At higher doses most of the seedlings developed neither of these roots. Development of the first pair of adventitious roots followed a similar sequence. Inhibition of the growth of these roots started to appear at 60,000 r, while complete inhibition was noted in some instances at 80,000 r. At 100,000 r usually only one member of the pair was present, and it was reduced in length. Seedlings with both members of the pair missing were rarely noted. In a few isolated instances, at the higher dose levels, failure of one of the first adventitious roots to develop was accompanied by the appearance of a small second adventitious root.

Curvature responses

Curve I in Figure 5 shows the average positive phototropic curvatures of 20 intact 96-hour seedlings that were exposed to 200 meter-

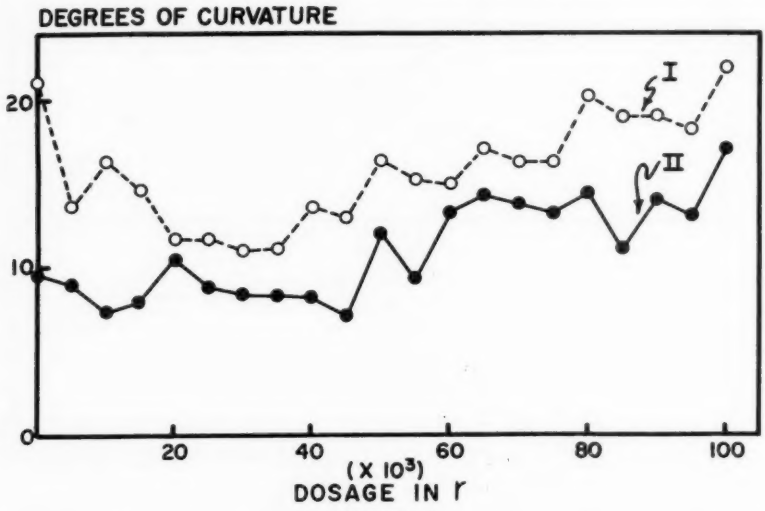

FIGURE 5

Tropic curvatures of *Avena* coleoptiles from humidified seeds which were exposed to gamma radiation. Each point represents an average of 20 or more measurements.

Curve I shows positive phototropic curvatures induced by 200 meter-candle-seconds of white light applied unilaterally.

Curve II shows curvatures induced by 30 μ amperes of direct current applied transversely for 2 minutes.

candle-seconds of unilateral white light from an incandescent lamp (Backus and Schrank, 1952). After the beginning of the exposure to light, the plants were allowed a period of 90 minutes for curvature to develop. The Judkins (1945) method was used to measure these and all subsequent curvatures. A positive phototropic curvature was observed over the entire dose range. Maximum inhibition of curvature was revealed when doses of 20,000 to 35,000 r were applied to humidified seeds. From 35,000 to 100,000 r an erratic increase in curvature is shown by Curve I. Coleoptiles from seeds exposed to 100,000 r develop about the same magnitude of curvature as the controls. It should be pointed out that 96 hour seedlings from seeds receiving 100,000 r reach an average length of only 15 mm., but they retain the ability to develop maximum positive phototropism.

The curvature responses to electrical stimulation are shown by Curve II in Figure 5. Direct current of 30 μ amperes was applied transversely for a duration of 2 minutes to a level of 2.5 mm. below the apex. Details of the current application have been described elsewhere (Schrank, 1948). Only slight inhibition of curvature was observed for doses up to 45,000 r. Above this dosage a gradual increase in the magnitude of curvatures was noted. For the higher doses a net stimulation of the electrically induced curvatures was obtained.

The geotropic curvatures of coleoptiles from gamma irradiated seeds are shown in Figure 6.. When the seedlings reached the age of 95 hours they were transferred to special lucite holders which contained Shive's solution. After one hour of rest in the individual holders, the seedlings were placed in the horizontal position for 90 minutes. The curvatures were measured as before. From 0 to 5,000 r the radiation was applied in increments of 500 r. With the exception of a few points, the curve shows a straight line increase of geotropic curvature with a maximum of 48° at 5,000 r. The magnitude of curvature is practically doubled, although the coleoptile length (see Figure 1) is slightly inhibited by this dose of radiation. From 5,000 to 40,000 r, geotropic curvature decreased to the minimum also manifested by the control coleoptiles. A similar decrease in oxygen consumption has been reported for doses between 4,000 and 10,000 r (Schrank, 1956). The magnitude of bending continued to increase from 40,000 to 103,000 r. Again it is relevant to note that coleoptiles which average only 15 mm. in length are geotropically more active than controls.

Discussion

Since little is known about the growth characteristics of plants grown from seeds which had been exposed to ionizing radiation, it is probably premature to attempt an extensive evaluation of the present results. There are, however, a few salient implications that should be considered.

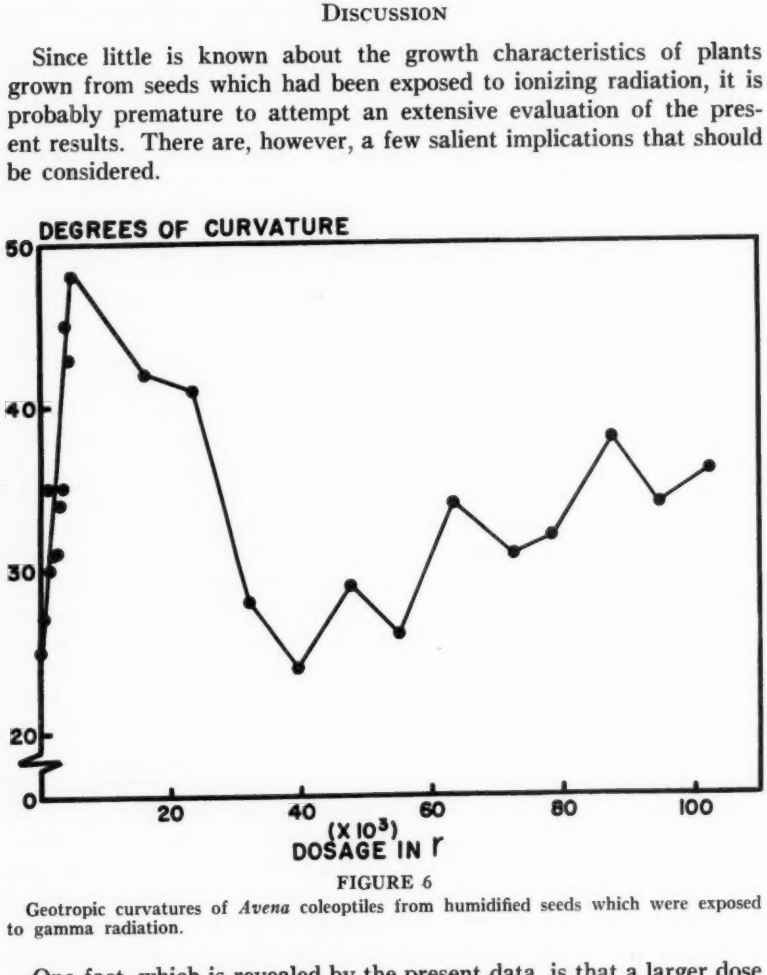

FIGURE 6

Geotropic curvatures of *Avena* coleoptiles from humidified seeds which were exposed to gamma radiation.

One fact, which is revealed by the present data, is that a larger dose of gamma radiation is required to prevent germination of the seeds than is necessary to inhibit the subsequent growth of roots and coleoptiles. It is also evident that the extent of the growth inhibition increases with increased doses. A similar relationship has been ob-

served when the radiation is applied directly to the growing tissue (Tanada, 1952).

Another significant observation was the fact that an increase in the moisture content of the seeds gave a marked protection against the growth inhibition effect of the higher doses. It seems unlikely that the inhibition of elongation was brought about indirectly by irradiation of the water for two reasons. (A) The water content of air dried seeds is very low. (B) A 10 per cent increase in weight due to water intake made the seeds less sensitive to the growth inhibition effects of the larger doses. Logically then, the observed effects of gamma radiation should be explained on the basis of the direct action theory (Dale, 1954). The effects of water on the radiation sensitivity of barley seeds, under somewhat different conditions, has been reported by Konzak (1955).

Coleoptiles from seeds receiving 100,000 r reach a maximum length equal to about one-third of the controls. Such irradiation effectively inhibits elongation, but these short coleoptiles manifest curvatures as large as or larger than controls of the same age. Curvatures induced by unilateral light, direct current and gravity all show the same general response. Young coleoptiles, 15 mm. in length, from non-irradiated seeds which still retain most of their elongation reserve, develop an average negative geotropic curvature of 40 degrees when they are kept in the horizontal position for 90 minutes. Thus it appears that coleoptiles, which are still short at the age of 96 hours due to irradiation, manifest tropic curvatures that are somewhat similar to the curvature responses of younger coleoptiles.

The large increase in the geotropic curvature caused by exposing the humidified seeds to 5,000 r (Figure 6) is another important aspect of the present study. Since these coleoptiles are inhibited only slightly in length, the increased curvature cannot be due to a growth reserve and remains as a stimulatory effect.

Summary

1. Doses up to 100,000 r of gamma radiation from radioactive Cobalt were applied to *Avena* seeds, and the effects on coleoptile length, root length and tropic curvatures of the seedlings were observed.

2. Germination of the seeds was not affected by maximum doses of 100,000 r.

3. Inhibition of root and coleoptile growth increased with increased irradiation. Roots and coleoptiles, at the age of 96 hours, from seeds receiving 100,000 r were inhibited from 65 to 75 per cent. Seedlings grown from seeds exposed to radiation at 1.2° and 13° C developed slightly less than controls.

4. Equilibration of seeds at 87 per cent relative humidity before irradiation resulted in marked decrease of the inhibitory effects induced by doses above 30,000 r.

5. Curvature responses to unilateral white light (200 meter-candle-seconds), direct current (30 μ amperes applied transversely for 2 minutes), and gravity (90 minutes in the horizontal position) of coleoptiles from seeds that had been exposed to 100,000 r were as large or larger than the curvatures of normal plants of the same age.

6. Geotropic curvature of coleoptiles from seeds exposed to 5,000 r were found to be twice as large as curvatures of coleoptiles from non-irradiated seeds.

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A STUDY OF THE YOLK PROTEINS OF FROG EGGS BY PHYSICAL AND CHEMICAL MEANS[1]

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Yolk platelets from frog eggs suspended in distilled water may be solubilized by the addition of 0.01 M $CaCl_2$. This involves the conversion of vitellin, *water insoluble protein* constituting the bulk of the yolk protein, to a material which in terms of solubility resembles livetin, *water soluble* protein occurring naturally in frog eggs. According to Flickinger (7), and Barth and Barth (1), the *in vitro* conversion is related to the action of phosphoprotein phosphatase which requires the presence of $CaCl_2$ or an acid pH for activity. It has been postulated by Flickinger that a similar mechanism may operate *in vivo;* that is, vitellin in the egg may be acted upon by the enzyme, the soluble product being naturally occurring livetin. This concept would gain support if it were shown that natural livetin, derived "livetin", and vitellin possess certain physical and chemical similarities. Accordingly, the methods of analytical ultracentrifugation, electrophoresis, and lipide analysis have been applied to such preparations to test this possibility, as well as to determine, for the first time, several physical and chemical properties of these molecules.

MATERIALS AND METHODS

Unlaid eggs of the Leopard frog, *Rana pipiens*, were washed and homogenized in distilled water, centrifuged at 2500 g and the supernatant discarded. The pigment-yolk residue was washed five times with homogenization and centrifugation, and was then treated in the following manner:

[1] This work was completed under Contract No. AT-04-1-GEN-12 between the Atomic Energy Commission and the University of California, Los Angeles, and was supported in part by a grant from the American Cancer Society.

Vitellin and livetin preparation. An equal volume of 20 per cent NaCl was added to the pigment-yolk residue. Yolk appeared to go into solution instantly, and was separated from the residual material by high speed centrifugation and filtration. Dialysis at 2°C against several changes of distilled water served to precipitate and to purify vitellin from the water soluble livetin.

Incubation supernatant (derived "livetin") preparation. Washed pigment-yolk at 0.01 M $CaCl_2$ concentration was incubated overnight at 32°C with shaking. This treatment solubilized protein from the yolk. In some cases vitellin alone was incubated with 0.01 M $CaCl_2$, a procedure which also resulted in solubilization.

Most of the ultracentrifuge studies were carried out with 10 per cent NaCl solutions (pH 6.0). Some measurements were made with 0.01 M $CaCl_2$ solutions (pH 5.9) and with 0.1 M glycine NaOH buffer (pH 11.3). The Spinco ultracentrifuge (Model E) was operated at 190,000 g. Solutions were of varying protein concentration so that extrapolations to infinite dilutions could be made. Corrections to standard conditions were applied according to Greenberg (8) and Svedberg (10).

Before electrophoresis, all materials were dialized overnight against 0.1 M glycine buffer (pH 11.3), centrifuged for 30 minutes at 15,000 g, and diluted with glycine buffer so that the solutions were approximately 1.4 per cent protein. All preparations were run singly, and in every possible combination of any two materials in the Perkin-Elmer electrophoresis apparatus (Model 38) for one and one-half hours at 10 ma (ice bath).

Analysis for total lipide was by the method of Delsal (3); extraction with 15 volumes of a 1:4 solution of methanol in methylal was followed by evaporation and re-extraction with three 10 ml volumes of redistilled petroleum ether (b.p. 60-70°C). Isolation of sterol esters, triglycerides, sterols, and phospholipides was by the chromatographic column technique described by Fillerup and Mead (6). Analyses for total phosphorus of the whole lipide and phospholipide fractions were by the methods of Elek (4, 5).[1] Values for total nitrogen were obtained by the Micro Kjeldahl technique.

[1] These analyses performed by Dr. Adelbert Elek, Elek Microchemical Laboratories, Los Angeles, Calif.

RESULTS

The ratio of livetin to vitellin in cells was observed to increase as the embryo developed from egg to tailbud stage.

Ultracentrifuge measurements indicated that oocyte vitellin suspended in 10 per cent NaCl (pH 6.0) sedimented as two discrete components. The largest of these components (approximately 95 per cent of the total protein) sedimented at an average extrapolated rate of 11.0 S.[2] The smaller component sedimented at a rate of 6.0 S. In 0.1 M glycine NaOH buffer solution (pH 11.3) other components were discernible (see Fig. 2).

Natural livetin from oocytes consisted of species sedimenting at average rates of 11.5 S, 16.4 S, and 19.2 S in 10 per cent NaCl. Additional components appeared when centrifugation was performed in 0.01 M $CaCl_2$ (pH 5.9) or 0.1 M glycine buffer. The proportions of the peaks varied from sample to sample even in 10 per cent NaCl and for the same sample the corrected sedimentation rates were generally greater in aqueous solution.

Derived "livetin" also displayed boundaries sedimenting at corrected rates of 11.0 S, 15.6 S, and 19.6 S in 10 per cent NaCl. Again there was considerable variability from sample to sample in the sizes of the respective boundaries. When centrifugation was performed in 0.01 M $CaCl_2$ or 0.1 M glycine buffer, the corrected sedimentation rates were also generally faster for the same sample. Other components were sometimes observed.

A single run was made in 10 per cent NaCl with vitellin which had been solubilized by addition of 0.01 M $CaCl_2$. This material displayed a major boundary very similar in proportion and sedimentation rate to vitellin. Faster sedimenting boundaries peculiar to vitellin incubation supernatant and natural livetin were present but in smaller proportions. The 6S component characteristic of vitellin was not seen. Centrifugation in 0.01 M $CaCl_2$ revealed similar boundaries but significantly faster sedimentation rates.

Some typical ultracentrifuge patterns are shown in Figs. 1 to 6, and individual measurements of sedimentation rates are plotted on graphs in Figs. 7 to 9 with extrapolation to infinite dilution.

Electrophoretic patterns showed one major peak in all of the different preparations. This peak had close to the same mobility toward

[2] These figures are significant to the decimal point.

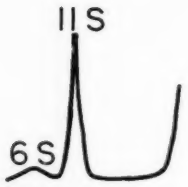

FIG 1

FIG 2

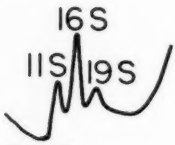

FIG 3

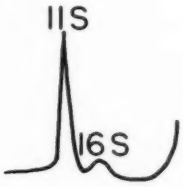

FIG 4

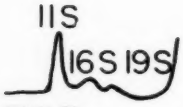

FIG 5

FIG 6

FIGURE 1

Ovarian vitellin. Ultracentrifuge pattern 80 minutes after start of run, 190,000 g. Medium 10% NaCl, pH 6.0. Protein concentration, 3.0%. Bar angle 60 degrees.

FIGURE 2

Same vitellin as for Fig. 1. Ultracentrifuge pattern 55 minutes after start of run, 190,000 g. Medium 0.1 M glycine—NaOH buffer, pH 11.3. Protein concentration, 1%. Bar angle 45 degrees.

FIGURE 3

Ovarian livetin. Ultracentrifuge pattern approximately 90 minutes after start of run, 190,000 g. Medium, 10% NaCl, pH 6.0. Protein concentration, 1.5%. Bar angle 20 degrees.

FIGURE 4

Tailbud stage livetin. Ultracentrifuge pattern approximately 90 minutes after start of run, 190,000 g. Medium 10% NaCl, pH 6.0. Protein concentration, 3.0%. Bar angle 60 degrees.

FIGURE 5

$CaCl_2$ treated vitellin. Ultracentrifuge pattern approximately 90 minutes after start of run, 190,000 g. Medium, 10% NaCl, pH 6.0. Protein concentration 1.3%. Bar angle 60 degrees.

FIGURE 6

Vitellin incubation supernatant. Ultracentrifuge pattern approximately 90 minutes after start of run, 190,000 g. Protein concentration 1.5%. Bar angle 60 degrees.

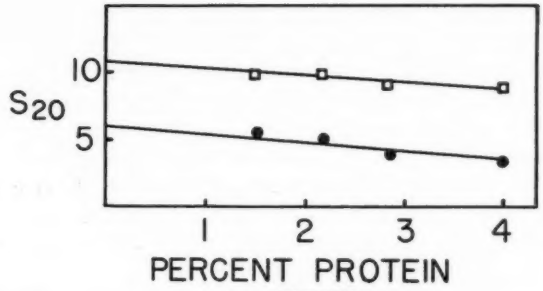

FIGURE 7

Extrapolation of corrected sedimentation constants of two components of vitellin in 10% NaCl to infinite dilution. The extrapolated values represent the sedimentation free from effects of protein interaction. Each point on these curves represents the value for a different sample.

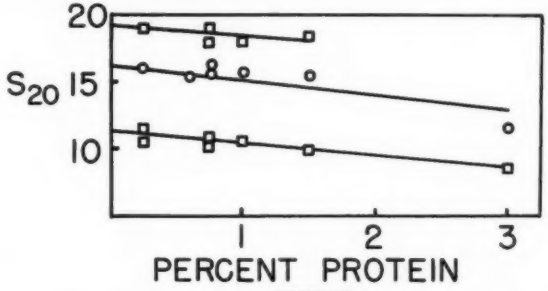

FIGURE 8

Extrapolation of corrected sedimentation constants of three components of livetin in 10% NaCl to infinite dilution. Each point on these curves represents the value for a different sample.

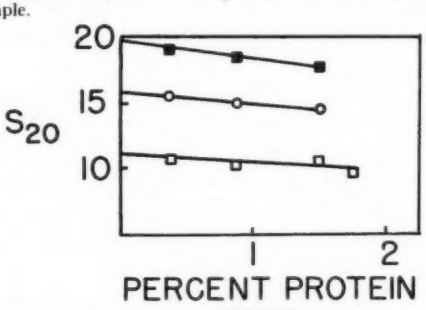

FIGURE 9

Extrapolation of corrected sedimentation constants of three components of derived "Livetin" (vitellin incubation supernatant and $CaCl_2$ solubilized vitellin) in 10% NaCl to infinite dilution. Each point on these curves represents the value for a different sample.

the anode in all cases ($3.5x10^{-4}$ for the ascending limb and $3.6x10^{-4}$ for the descending limb). In some cases vitellin displayed small peaks migrating ahead of the major boundary and in all cases vitellin and livetin showed small peaks migrating behind the largest boundary, but these peaks moved at different rates in the various preparations, hence their interpretation is difficult. Mixtures of the above materials showed a single major migrating boundary. Patterns of typical runs, in this case the descending limb of a solution of ovarian vitellin and also the descending limb of a mixture of ovarian livetin and vitellin are shown in Figs. 10 and 11.

FIG 10

FIG 11

FIGURE 10
Ovarian vitellin with equal amount of ovarian livetin. Descending limb. Movement of boundaries is to the right. Protein concentration, 1.4%.

FIGURE 11
Ovarian vitellin. Descending limb. Movement of boundaries is to the right. Protein concentration, 1.4%.

Comparison of the total lipide and the total nitrogen of vitellin, natural livetin, and derived "livetin" showed that, despite differences in sedimentation patterns, the ratio of lipide to protein in vitellin and derived "Livetin" (Table I) was similar (approximately 1:4). The ratio of lipide to protein was only slightly less in the case of natural ovarian livetin. Further, chromatographic column analyses and lipide phosphorus analyses indicated that 75 to 85 per cent of the total lipide was present as phospholipide in all three molecular types.

Discussion and Conclusions

The ultracentrifuge experiments, as carried out in 10 per cent NaCl solution, clearly showed that vitellin, natural livetin, and derived

"livetin" (incubation supernatant and $CaCl_2$ solubilized vitellin) each consisted in part of a molecular species which sedimented at approximately 11 S. In the case of vitellin, $CaCl_2$ solubilized vitellin, and livetin from embryos in the tailbud stage (Figs. 1, 4, and 5) this com-

TABLE I
Percentages of Lipides in Vitellin, Livetin, and of derived "Livetin".

Material	No. of Experiments	Total Lipide	Sterol Ester[1]	Trig.[1]	Sterol[1]	Phospho. Lipide[1]
Vitellin (Oocyte)	2	18.9 (18.3-19.6)	8.2	12.3	5.5	74.0[2]
Vitellin (Tailbud)	1	15.4	—	—	—	—
Livetin (Oocyte)	2	14.0 (12.0-16.0)	5.0	14.0	8.0	73.0
Livetin (Tailbud)	1	8.3	—	—	—	—
$CaCl_2$ Vitellin (Oocyte)	1	20.0	8.2	12.3	5.5	74.0
Incubation Supernatant (Oocyte)	3	19.9 (18.9-21.9)	2.9	9.1	2.9	85.1[2]

[1] These percentage values are referable to the total amount of lipide present in the fraction.

[2] Results of a single chromatographic separation. These results fortify each other, however, since the lipide component of vitellin would not be expected to change radically during incubation.

ponent was the major one. Electrophoretic patterns (Figs. 10 and 11) indicated an even more striking similarity of these materials. Lipide analyses (Table I) also confirmed the notion of essential similarity but livetin, especially from the embryo in the tailbud stage, did contain less total lipide.

Aside from the similarities mentioned above, the differences among these preparations may possibly be useful for a more complete hypothesis of the phenomena involved in the conversion of vitellin to livetin. For example, it was noted that in the case of vitellin a slower sedimenting component (approximately 6 S) was present which was not found in preparations of either natural livetin or derived "livetin". Also, in some of the electrophoretic patterns obtained with vitellin, a small amount of a faster migrating component was resolved. The presence of these components recalls the suggestion of Barth and Barth (1) that vitellin is a complex of at least two protein types. Ac-

cording to these authors, only one of these (present in lower concentration) is a phosphoprotein. The action of phosphoprotein phosphatase is to dephosphorylate this protein thus splitting the complex so that the nonphosphoprotein species may go into solution. Whether the 6 S or fast migrating components are actually such complexing agents is a matter for further investigation.

Flickinger (7) has proposed not only that vitellin is converted directly to livetin by dephosphorylation but also that the livetin fraction possesses phosphoprotein phosphatase activity. Moreover, he stresses the possibility that the major, and not the minor, component of vitellin is the phosphoprotein and that upon dephosphorylative conversion to livetin the amount of phosphate lost by the molecule is insufficient to affect the sedimentation rate. One of his objections to the scheme proposed by Barth and Barth is that the minor component, described by these authors, should differ in migration rate from the major component when it is dephosphorylated; but it did not. Flickinger does, however, admit the possibility that the small so-called phosphoprotein component could have melded with the large component in such patterns.

Although one or both of the above schemes may be compatible with the similar electrophoretic behavior of the major components of glycine-NaOH solubilized vitellin, natural livetin, and derived "livetin", they do not seem to account for the additional faster sedimenting species seen in natural livetin and derived "livetin". Since changes in corrected sedimentation rate, as well as actual amounts of these "extra" components, could be varied by changing the media with which centrifugation was carried out, the possibility exists that they may be aggregates of a smaller species, perhaps one quite similar to the predominant unit of the fraction which sedimented at 11 S. Only a small per cent of the alleged aggregates seemed to be readily split by high NaCl concentration. Thus, association of molecules by lipid to lipid bonding is suggested. This idea is supported by two other observations: (A) Livetin obtained from the embryo in the tailbud stage apparently contained less than half the lipide found in oocyte livetin and also failed to show "aggregate formation". (B) The "aggregates" migrated for the most part at the same rate as did the 11 S species, indicating that the surface charges were fairly similar.

Biological Significance: The biological significance of the observations discussed in the preceding paragraphs is by no means clear, but

the following tentative scheme may be useful as a working hypothesis:

The increasing ratio of livetin to vitellin during embryonic development strongly suggests that vitellin in the frog egg is the source of materials for several important growth and differentiation processes. The first such substance to be made readily available to further metabolic mechanisms is inorganic phosphate which is stripped from vitellin by phosphoprotein phosphatase. This phosphate has been shown to appear in the newly synthesized nucleic acids of the frog egg (9) and is undoubtedly also incorporated into adenosinediphosphate and adenosinetriphosphate (2).

The large amount of phospholipide associated with vitellin is a major potential store of energy for the developing embryo (1). Here again, phosphoprotein phosphatase seems to play a major role since insoluble vitellin apparently becomes solubilized by the dephosphorylative action of the enzyme, taking the form of livetin. Thus the phospholipide and other lipides are made directly available to specific fat hydrolizing enzymes.

The residual protein may be hydrolized to smaller peptides or amino acids and then either utilized for energy or resynthesized into the cytoplasmic proteins. It is also possible that yolk protein, or high molecular weight peptides, may be merely modified to form cytoplasmic proteins (7).

Summary

Ultracentrifugal, electrophoretic, and chemical properties of vitellin, natural livetin, and derived "livetin" (from frog eggs and embryos) have been studied. Similarity of the main components of the yolk proteins, vitellin and livetin, has been demonstrated. The data are interpreted as evidence for the conversion of vitellin to livetin during embryonic development. Further evidence for such a conversion is seen in the change towards a higher livetin to vitellin ratio in the cells as the embryo developed.

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SERIAL SEQUENTIAL DEVELOPMENT OF ANOMALOUS METACARPAL AND PHALANGEAL OSSIFICATION CENTERS IN THE HUMAN HAND*

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An earlier paper from this clinic concerning the effect of dietary supplements on child growth and development was devoted to an evaluation of the frequency and types of anomalies in the hand skeleton of 266 white children (14). The present report, representing a serial rather than a cross-sectional study, describes the sequence of changes noted in the anomalous metacarpals and phalanges. The findings are derived from an examination of serial roentgenograms of the left hand of each of the 266 children taken at intervals of three to six months.

A skeletal structure represented by a roentgenographic shadow was termed an anomaly if it resembled an ossification center except that it was located in an abnormal site; if it was abnormally oriented; if it was markedly small in comparison with neighboring skeletal segments; and/or if it was distorted. The metacarpal and phalangeal anomalies were subgrouped into supernumerary (isolated) centers of ossification, pseudo-epiphyses, and notches. The last embraced four types or categories (14).

Sequence of Changes in Anomalous Metacarpal and Phalangeal Ossification Centers: The stages in the development of an isolated center of ossification are illustrated in Figure I. At 2 months of age (Fig. I-A), the future site of development of this center is adjacent to the slightly concave proximal margin on metacarpal II. Progressive deepening of the concavity is seen in the roentgenograms made at 7 months and at 1 year. In the latter (Fig. I-C) a completely inde-

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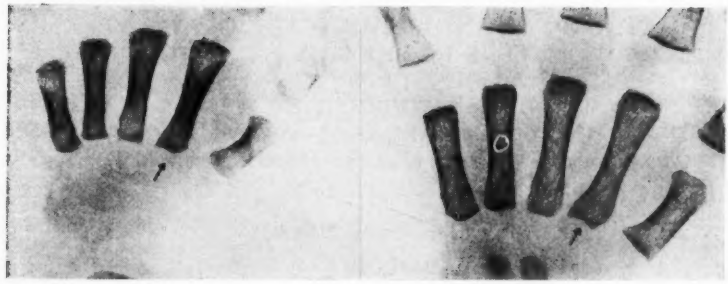
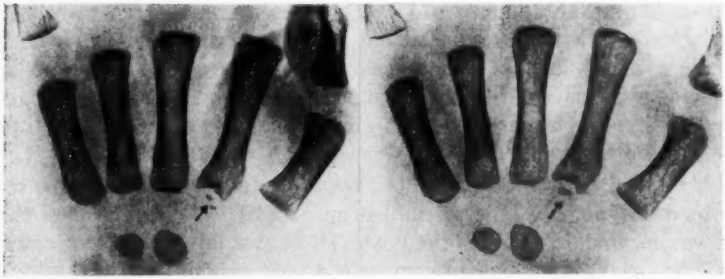


I-A, 2 months I-B, 7 months



I-C, 1 year I-D, 1 year 4 months

I-E, 1 year 7 months I-F, 2 years 1 month

FIGURE I
Sequential Development of Supernumerary Epiphysis at Distal End of Metacarpal II in Boy #1468

pendent center of ossification is present. As shown in Figures I-D and I-E, the center continues to grow and differentiate. By age 2 years 1 month (Fig. I-F), the space between the proximal end of the shaft of metacarpal II and the independent center is bridged by a bony spur. Presumptively, this center will fuse with the shaft preparatory to the attainment of the adult contour.

The pseudo-epiphysis group is comprised of several types. Among the most distinctive is that represented in Figure II which depicts the sequential changes in the hand skeleton of a boy between the ages of 4 years and 14 years 4 months. In the roentgenogram taken at 4 years (Fig. II-A) a relatively small discoidal mass of bone is present at the center of the slightly concave distal end of metacarpal I. The demarcation between the shaft and the discoidal pseudo-epiphysis is more distinct at 5 years 11 months (Fig. II-B). The distal end of the shaft has become more concave and the pseudo-epiphysis is attached apparently by a very short pedicle. Radialward, a narrow rectanguloid, proximally directed notch (or fissure) separates the shaft from the pseudo-epiphysis. By 7 years 7 months (Fig. II-C) the radialward portion of the pseudo-epiphysis shows differential thickening and the fissure has altered to a narrow somewhat crescentic sulcus, convex proximally, which delimits the pseudo-epiphysis from the shaft. Union between shaft and the periphery of the pseudo-epiphysis, progressing ulnoradialward, is complete in Figure II-E at age 14 years 4 months. A similar pseudo-epiphysial development is seen at the distal end of the proximal phalanx of the thumb of this boy.

A more striking example of a pseudo-epiphysis is presented in Figure III. In this film, taken when the boy was 8 years 3 months, a central inverted cone of osseous tissue, emerging from a corresponding concavity on the distal end of metacarpal I, terminates in a mushroom-like cap which is slightly narrower than the width of the shaft. A non-osseous, i.e., cartilaginous, interval separates the cap from the peripheral portion of the diaphysis. The same structures at 6 years 6 months, when the first roentgenogram was made, differ only in the slightly narrower diameter of the cap relative to the shaft. Similar rates of growth preserve this relationship until 9 years 3 months when the diameter of the cap and shaft are approximately equal. At 10 years 3 months, synostosis of shaft and pseudo-epiphysis begins and advances to almost complete fusion at 14 years 9 months (Plate

II-A, 4 years

II-B, 5 years 11 months

II-C, 7 years 7 months

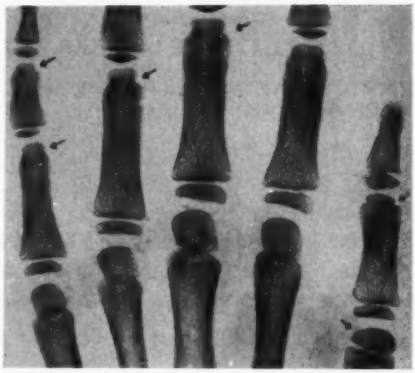
II-D, 11 years 1 month

II-E, 14 years 4 months

FIGURE II

Sequential Development of Pseudo-Epiphyses in Metacarpal I and Proximal Phalanx of Thumb, Notched Metacarpals II and V (Boy #51)

I-A). The progression toward maturity is similar to that shown in Figure II, including differential thickening of the radialward margin of the pseudo-epiphysis. Variation in age at which fusion is completed is demonstrable: terminated at 14 years 4 months for the boy represented in Figure II, but incomplete at 14 years 9 months for the



III, 8 years 3 months

FIGURE III
Pseudo-Epiphysis Metacarpal I, Notched Proximal Phalanges Digits 1, 3, 4, 5, Middle Phalanx Digit 5 in Boy #174

one in Figure III and Plate I-A. A similar, but less distinctive, pseudo-epiphysial development is shown at the distal end of the proximal phalanx of the thumb of each of these boys (Figs. II, III). Less sharply demarcated differentiation of phalangeal pseudo-epiphyses, involving the proximal phalanges of the fingers and the mesophalanx of the little finger, appears in Figure III.

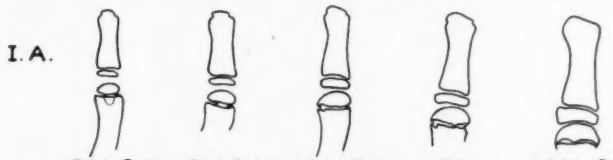
The types of pseudo-epiphyses on metacarpal I, described above, are more apparent than the majority of those found at the proximal ends of metacarpals II through V. The development of a pseudo-epiphysis at the proximal end of metacarpal II over a period of 5 years 10 months is shown in Figure IV and Plate II-A. At 1 year (Fig. IV and Plate II-A-1), the proximal end of metacarpal II is notched slightly at both the radial and ulnar margins. At 2 years 3 months (Plate II-A-2), the notch on the ulnar margin has the appearance of a reversed, inverted, roughly italic "L", whereas the notch on the

radial side shows little change. By 2 years 11 months (Plate II-A-3), the notch on the ulnar margin appears as a short, horizontal rectangular fissure, open at its ulnar extremity. A faint, irregular narrow line of increased radiopacity extends from the radial end of this fissure to the distal extremity of the inverted "L"-shaped notch on the proximo-

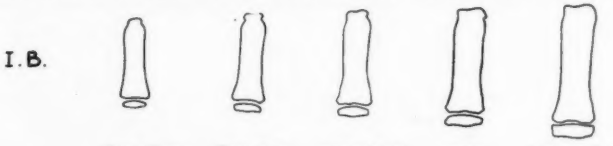
PLATE I

SEQUENTIAL DEVELOPMENT OF ANOMALIES

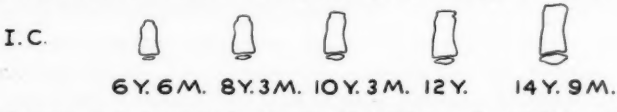
PSEUDO-EPIPHYSIS METACARPAL I AND NOTCHED PROXIMAL PHALANX OF THUMB (BOY NO. 174)



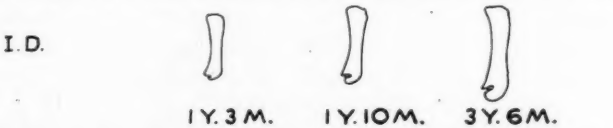
NOTCHED PROXIMAL PHALANX 4TH DIGIT (BOY NO. 174)



NOTCHED MESO-PHALANX OF 5TH DIGIT (BOY NO. 174)



PSEUDO-EPIPHYSIS METACARPAL II (BOY NO. 1443)



PSEUDO-EPIPHYSIS METACARPAL I (BOY NO. 313)

See Plate II-A for continuation of pseudo - epiphysial development of metacarpal II in this boy.

IV, 1 year

FIGURE IV
Initial Stage in Development of Pseudo-Epiphysis at Proximal End of Metacarpal II in Boy #517

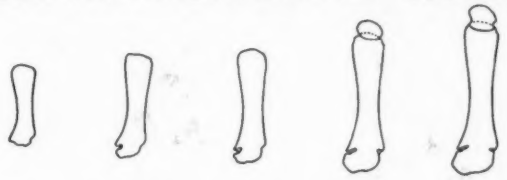
radial margin of metacarpal II. Continued ossification expands the base of the metacarpal proximal to these notches (Plate II-A-4 and II-A-5, ages 5 years 5 months and 6 years 10 months, respectively). The progression may be observed further in Figure V which represents the hand skeleton of a girl from age 6 years 2 months to age

PLATE II

SEQUENTIAL DEVELOPMENT OF ANOMALIES

PSEUDO-EPIPHYSIS METACARPAL II (BOY NO. 517)

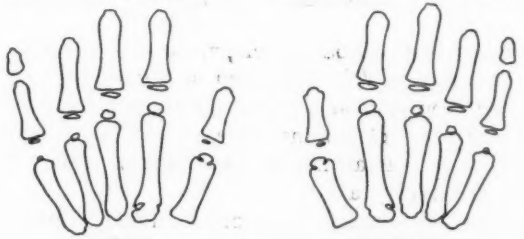
II.A.



1 Y. 2Y. 3M. 2Y. 11M. 5Y. 5M. 6Y. 10M.

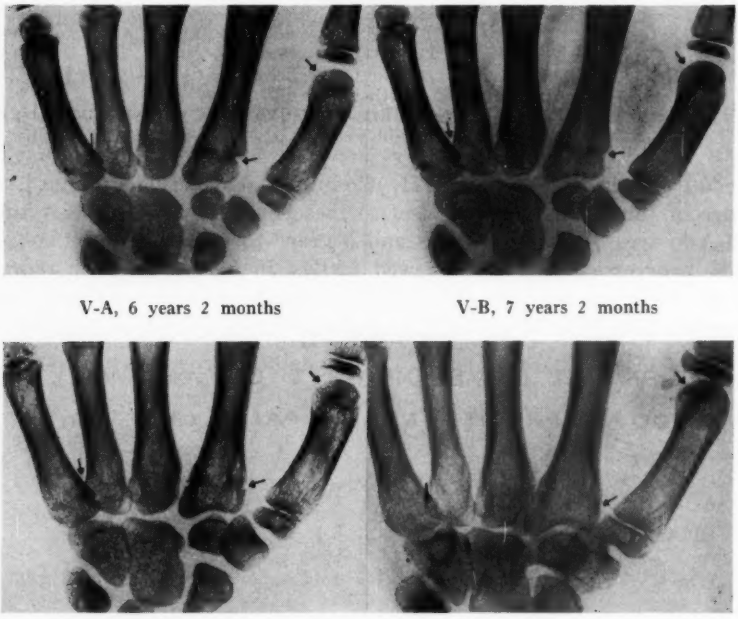
BILATERAL OCCURRENCE OF ANOMALOUS CENTERS IN A 6 YEARS, 6 MONTHS OLD BOY (BOY NO. 2)

II.B.



LEFT RIGHT

11 years. The sequence is such that the notches on both the radial and ulnar border become more and more horizontally oriented. By 11 years, the notch on the radial side is completely ossified while a faint trace is discernible on the ulnar margin.



V-A, 6 years 2 months V-B, 7 years 2 months

V-C, 7 years 11 months V-D, 11 years

FIGURE V
Sequential Development of Pseudo-Epiphyses in Metacarpals I and II and Bilateral Notch, Metacarpal V (Girl #379)

A similar but less obvious development of a pseudo-epiphysis occurring on metacarpal V is illustrated in Figure V. In this instance, the very short, approximately rectangular notch on the ulnar margin disappears at 7 years 11 months while the obliquely inclined proximo-distal notch on the radial margin, seen faintly at 9 years 6 months, is fully ossified at 11 years.

In some cases, ossification of the proximal end of metacarpal II and occasionally metacarpals III through V begins by the parasagittal ex-

tension of a relatively narrow or wide band of ossified tissue into the cartilage. The band may expand in such fashion that a notch develops on only one border as represented in Plate I-D. The narrow, rectangular, almost horizontal notch is clearly seen at 3 years 6 months but has been practically obliterated 6 months later.

Less conspicuous evidences of pseudo-epiphyses are typified by the inverted "L"-shaped notch on the proximo-radial margin of metacarpal II and by the reversed roughly "L"-shaped notch on the proximo-ulnar margin of metacarpal V (Fig. VI). The transitory development of pseudo-epiphyses on metacarpals II and IV is represented in Figure VII. The rarity of pseudo-epiphyses on these two metacarpals as noted in our earlier report, conforms with the observations made by others (1)(16).

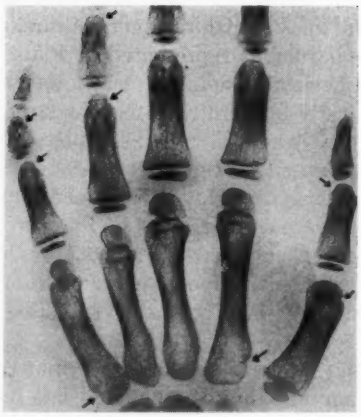
The pseudo-epiphyses tend to develop symmetrically. Plate II-B represents roentgenograms of the right and left hand of a boy, aged 6 years 6 months, both of which show pseudo-epiphyses on metacarpals I and II, on the proximal phalanx of the thumb and of the fingers and on the mesophalanx of the little finger.

Discussion

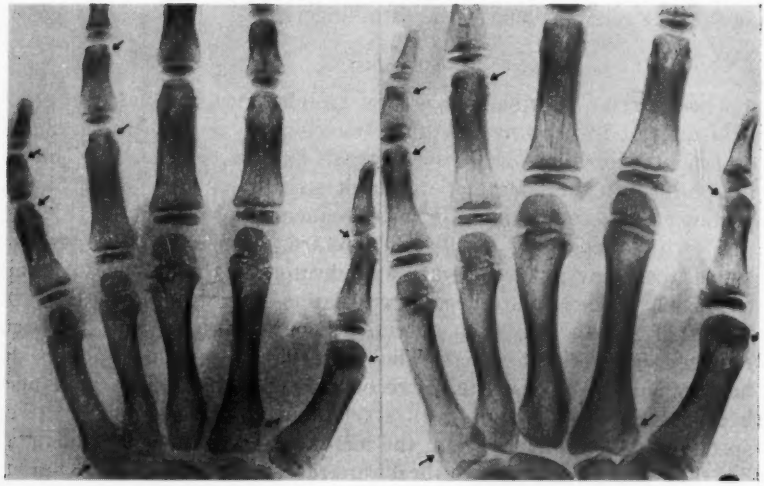
The material described above is of an inherent as well as of a practical interest in showing that these developmental (growth + differentiation) aberrations in the metacarpals and phalanges usually metamorphose into the characteristic adult structures. The practical importance of these anomalies is their frequent occurrence (1)(12)(15)(16) with almost any agent which retards or arrests development; hence, they may serve the wary as indicators in the absence of clinical signs. Their occurrence in association with transverse lines of "arrested growth" has been observed in our series (4) and has been reported by Weinert (16). The presence of pseudo-epiphyses in healthy children is a matter of record (2)(11)(15). They have also been described in some cases of accelerated growth (12) (15). Irrespective of the etiologic agent, the metacarpal and phalangeal anomalies differentiate in one of several patterns (forms) which are characteristic for that center or group of centers. One infers, therefore, that some mechanical factor or factors influence the development in each category.

The basic characteristic in the development of pseudo-epiphyses (Plates I-D and II-A), as distinguished from isolated centers of ossi-

Note brachymesophalanx and radialward clinodactylia of little finger.



VI-A, 7 years 8 months



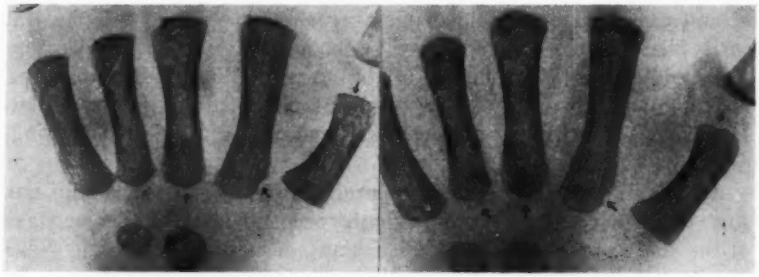
VI-B, 10 years 8 months

VI-C, 13 years 6 months

FIGURE VI

Sequential Development of Pseudo-Epiphysis, Metacarpal I, Notched Metacarpals II and V and Notched Phalanges on Digits 1, 4, 5 (Boy #553)

fication which yield supernumerary epiphyses, is the apparent growth and morphologic differentiation of a bud- or shoot-like extension of the shaft as ossification of the cartilage proceeds. Concomitant with this seeming extension from the shaft is the production of a limited variety of clefts or notches. These may be summarily classi-



VII-A, 1 year 6 months VII-B, 2 years 1 month

VII-C, 2 years 7 months VII-D, 3 years 3 months

FIGURE VII
Sequential Development of Notches on Metacarpals II, III and IV and Pseudo-Epiphysis on Metacarpal I in Boy #1309

fied, on the basis of location and form, into the following categories:

a) Parasagittal notch, usually approximates a right angle on either the radial or the ulnar margin of the end of the shaft. The short limb of the notch is generally perpendicular to the long axis of the bone. (Plate II-A-3, metacarpal II, radial margin. Fig. II-A, metacarpal V, ulnar margin.)

b) A more or less acute-angled notch, situated similar to *a*. The short limb of the notch is usually more or less perpendicular to the

long axis of the bone while the long axis slopes toward the sagittal axis of the shaft; hence, the axis of the angle is directed obliquely —distally in metacarpals II through V, but proximally in metacarpal I. (Fig. V-A, metacarpal II, radial margin.)

c) A somewhat obtuse-angled notch with its orientation and position similar to *a*. Often the type on the radial margin of metacarpal II and on the ulnar margin of metacarpal V, but characteristic of an early phase in the development of pseudo-epiphyses on the proximal and middle rows of phalanges of the fingers. (Fig. II-B, metacarpal II, radial margin. Fig. VI-A, proximal phalanges of fingers. Plate II-A-1 and 2, metacarpal II, radial margin. Plate I-B-1 proximal phalanx of fourth finger.)

d) Rectanguloid or somewhat paraboloid (C-shaped), usually narrow, notch with its long axis roughly perpendicular to the long axis of the shaft. (Fig. III, proximal phalanx of third finger. Plate I-A-3, metacarpal I. Plate I-C-4, mesophalanx of little finger.)

e) Shape similar to *d*, but with long axis of notch directed more or less obliquely. (Fig. VI-A, metacarpal I, radial margin. Plate I-D-3, metacarpal II, ulnar margin.)

f) Crescent-like narrow sulcus or groove, convexity proximalward, extending ulnarward from disto-radial margin of metacarpal I. (Fig. II-D, metacarpal I.)

The most common sequential change is the alteration of types *a*, *b*, or *c* into type *d* (occasionally, *b* into *e*) and their ultimate obliteration as differentiation and ossification of the cartilaginous end of the shaft gradually alters the shaft toward its adult size and configuration. Type *a*, when present on the radial side of metacarpal II or on the ulnar side of metacarpal V, often disappears—without changing to type *d*—as the result of continued ossification parallel to the length of the notch. The type *c* (obtuse angle) notch, which characterizes an early phase of pseudo-epiphysial development on the proximal and middle phalanges of the fingers, when bilateral constitutes the so-called flask-shaped termination of the phalanges. Its alteration to type *d* results from the radialward and ulnarward expansion of the distal extremity of the ossifying sagittal core of each phalanx.

The shallowness of notches of types *a*, *b*, and *c* relative to the width of the shaft, especially on the phalanges, often renders the question of the presence or absence of a pseudo-epiphysis a matter of definition. The occurrence of such shallow notches on the disto-radial

margin of metacarpal I or on the proximo-ulnar margin of metacarpal V, when indication of other pseudo-epiphyses is lacking, may represent a variation of the normal (16). The frequent association of these shallow notches with pseudo-epiphyses suggests, however, that the classification of these variations as pseudo-epiphyses is valid (12) (15). The presence of separate epiphyses at the distal end of the proximal and middle phalanges has been reported by Brailsford (1).

The transformation of the obtuse angle notch (type *c*) into the short rectangular or, alternatively, C-shape notches on the proximal and middle rows of phalanges is probably associated with the development of the circular foveae (or pits) on the radial and ulnar sides of the head. The foveae are the sites of attachment of the strong collateral ligaments securing the head of the proximal phalanx to the radial and ulnar margins of the base of the distal phalanx.

The ossification of the bilateral cleft delimiting the mushroom-like pseudo-epiphysis, when present on metacarpal I (Figs. III, II-C and D) to a crescentic-like sulcus has been noted above. Faint traces of such a groove or fissure on metacarpal I, or of a re-entrant (i.e., sistroid) notch on the disto-radial margin of metacarpal I (Fig. V-C), when noted in a single roentgen film may be the only indication of a former pedunculated pseudo-epiphysis.

The serial advance in the ossification of an isolated center of ossification, and its ultimate synostosis with the diaphysis, on metacarpal II of a boy from the age of 7 months to 1 year 7 months is illustrated in Figure I. The impending development of a type *d* notch is indicated in Figure I-F. The possible development of a type *c* notch on the proximo-radial margin of this metacarpal is foreshadowed in all six films. Additionally, in Figure I-C the proximal phalanx of the thumb and in Figure I-F the thumb metacarpal are developing (though not apparent in the half-tone) pseudo-epiphyses—discoidal bud on metacarpal I—consequent to the seeming extension of ossification from the respective diaphyses. Both the isolated supernumerary and the bud or mushroom-like extension of ossifying tissue from the diaphysis were observed also by Weinert (16) to occur in the hand of the same individual. Earlier, each of these modes had been viewed as *the* way by which pseudo-epiphyses develop; Siegert advanced the hypothesis of development from an isolated center and Hasselwander that of extension of ossification from the diaphysis (16).

The comparatively few basic types of notches or clefts represented in the pseudo-epiphyses and the similarity of those of the same segment of the hand in different individuals of both sexes may be compared with the similarity in type of cartilage canals (6) in all the epiphysial cartilages of man. Such canals, traversed by nutritive vessels, appear as early as the third fetal month and by the seventh fetal month (9) all of the larger epiphyses show a well-developed system of vascular canals. The entering angle of the vessels into the cartilage (9) tends to be perpendicular to the surface of the cartilage. The topographic arrangement of the cartilage canals, as depicted by Haines (6), suggests that they probably are consequent (or respond) to the same forces as those which control the direction of the nutrient canal in long bones. Both Hughes (7) and Schinz and his associates (13) agree that the direction of this canal probably is the resultant of the forces exerted by the growing bone and the growing blood vessels. Whether the canals are formed concomitantly with the inclusion of the perichondral vessels as the cartilage enlarges by superficial deposition (6) or whether the endothelium of the entering vessels has a chondrolytic action (8) remains a question. Parenthetically, the independent development of the normal epiphyses with respect to the diaphysis is shown by those congenital deformities in which the epiphyses ossify almost normally while the diaphysis fails to ossify (3).

The distribution of foramina for blood vessels supplving the segments of digits is similar regionally to the epiphysial sites. Thus, in the adult hand, the foramina generally are present (10) around the base of the metacarpals and at the junction of the head with the diaphysis. The shaft of many metacarpals does not show a nutrient foramen. On the proximal phalanges, however, a nutrient foramen occurs frequently (10) on the volar surface at the junction of the distal third with the proximal two-thirds of the bone. Constant foramina also develop on the volar surface at the junction of the head with the diaphysis in these proximal phalanges. The volar surface of the middle phalanges, just proximal to the trochlear surface, has very small foramina. With the nutrient foramina situated in protected positions, beneath the attachment of tendons, the blood supply is shielded when the tendons are in action.

The greater frequency of pseudo-epiphysial anomalies on the radial side than on the ulnar side of the hand, and the markedly lesser fre-

quency on metacarpals III and IV, is indicated in our earlier paper (14) and accords with observations by others (16)(1)(11). In a series of 200 children, 4 to 8 years of age, Posener and associates (11) found pseudo-epiphyses in the foot occurred almost exclusively on metatarsal I; metatarsals II through IV showed none and metatarsal V had them in only 1 per cent of the cases. This localization in both hand and foot suggests that the most active areas are those most likely to manifest developmental aberrancies such as pseudo-epiphyses.

Etiologically, one may consider that this greater regional activity—in the presence of factors retarding development—merely prolongs, and perhaps slightly exaggerates, a type of structure which is characteristic of normal development (12). Normally, the cartilage canals have a relatively ephemeral existence (6). The inconstancy and early disappearance of pseudo-epiphyses, often before even the advent of the center for the normally occurring epiphysis of an affected digital segment, tends to support the hypothesis that agents which retard normal development provide the conditions under which pseudo-epiphyses may be manifested. The retardation or inhibition of ossification represented by pseudo-epiphyses may be associated with the tensions and pressures exerted in the region of each segment showing such anomalies. The *in vitro* studies by Glucksmann (5) demonstrated that if a piece of ossifying cartilage be subjected directly to tensional forces, ossification is increased while reducing the tension correspondingly retards ossification.

Summary and Conclusions

1. The sequential alteration of developmental metacarpal and phalangeal anomalies as recorded by serial roentgenograms of the left hand of 266 children, aged 2 months to 14 years 9 months, was studied. The anomalies have been grouped into two principal categories: supernumerary centers of ossification and pseudo-epiphyses. Each type undergoes alteration and ultimately disappearance as differentiation progresses toward the shape and size characteristic of the adult bone.

2. These anomalies are inherently interesting variations in human development. Although they have been reported in some healthy children, their known association with malnutrition and diseases affecting osseous tissue should alert the clinician in the absence of overt signs of these conditions.

3. The repetition of basically the same few types of pseudo-epiphyses among affected children suggests the influence of mechanical factors in the production of such anomalies.

4. The types of notches and clefts resemble the patterns apparent in the vascular canals developed in the epiphysial cartilages during foetal life.

5. The greater frequency of pseudo-epiphyses on the medial and lateral borders of both hand and foot, i.e., the regions of greater activity in each member, together with the above mentioned resemblance to patterns of vascular canals developed in epiphysial cartilage during foetal life, suggests that influences which retard or inhibit growth merely prolong, and possibly exaggerate, structural features characteristic of normal development.

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INFLUENCE OF STYRYL QUINOLINE COMPOUNDS ON THE GROWTH OF SARCOMAS NATIVE IN RATS OF THE WISTAR INBRED STRAIN[1]

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In earlier studies (1, 2), it was found that oral administration of 4-(p-dimethylaminostyryl) quinoline, 4-(p-dimethylaminostyryl) quinoline methiodide and 4-(p-dimethylaminostyryl) quinoline methochloride brought about complete absorption of lymphoma #8 and in many instances of adenocarcinoma #11 growing in rats of the Wistar inbred strain. On the other hand, none of the compounds exhibited the same type of tumor-destroying activity against sarcomas growing in rats of the same strain, or even against sarcomas growing in the same rat bearing a lymphoma which regressed during the treatment (3, 4, 5). Oral administration of the styryl quinolines did, however, bring about considerable retardation of the growth of the sarcomas. Since the extent of retardation was, in some instances, as great as or even greater than that described by investigators utilizing other tumor-inhibiting agents, it seemed of moment to give a brief report of the results.

MATERIAL AND METHOD

Three 100 per cent transplantable sarcomas (#4, #10, and #13) which arose in rats of the Wistar inbred strain were available for these studies. Rhabdomyosarcoma #13 arose spontaneously in 1954; spindle cell sarcoma #4 and fibrosarcoma #10 were induced by means of methylcholanthrene in 1952 and 1950 respectively. Each of the sarcomas exhibited its own rate of growth; #4 being most rapid, #10 somewhat less rapid and #13 the slowest. This variation in rate of growth produced tumors of three sizes in rats implanted with tumor

[1] This investigation was supported in part by research grant C-285(C7) from the National Cancer Institute, Department of Health, Education and Welfare and in part by grants from The Medical Research Foundation and the Pew Memorial Foundation.

tissue at the same time; large sarcomas #4 (60 to 100 grams), medium size sarcomas #10 (40 to 60 grams) and small sarcomas #13 (20 to 30 grams). Rats which had been implanted with tumor tissue 8 to 10 days earlier and bore easily palpable tumors were selected for this study.

The anti-tumor properties of 122 new compounds[2] were tested against lymphoma #8 and in a few instances, against one or more sarcomas. Eleven of them exhibited lymphoma-destroying properties in that oral administration of food containing the compounds to rats bearing lymphoma #8, brought about complete absorption of the neoplasm in a high percentage of the treated animals. The eleven substances found to have anti-lymphoma activity were:

Code	
*2M2O	2-(p-Dimethylaminostyryl) quinoline
*2M2OALC	p-Dimethylaminophenylquinadylcarbinol[3]
*4M2O	4-(p-Dimethylaminostyryl) quinoline
*4M2M	4-(p-Dimethylaminostyryl) quinoline methiodide
4M2E	4-(p-Dimethylaminostyryl) quinoline ethiodide
4M2MCl	4-(p-Dimethylaminostyryl) quinoline methochloride
*4M2OZN	(4-Dimethylaminostyrylquinoline)$_2$ ZN
*44MORO	4-p-(4-Morpholyl) styrylquinoline
*4E2O	4-(p-Diethylaminostyryl) quinoline
4E2E	4-(p-Diethylaminostyryl) quinoline ethiodide
*4E2OCl	4-(p-Diethylaminostyryl) quinoline hydrochloride

Eight[4] of the most active of the eleven anti-lymphoma compounds were selected for use in the present study. Codes representing the compounds were used in place of the chemical names in the tables and wherever possible in the text.

The purpose of this study was to compare the size (weight) of tumors growing in rats treated with the eight anti-lymphoma substances, with the size of the tumors present in untreated rats at terminal stage, which varied for lymphoma #8 from 16 to 21 days, and for the sarcomas from 20 to 30 days, after implantation of the neoplastic tissue.

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[2] Fifty-four of the compounds were synthesized by C. T. Bahner; 42 were obtained from Eastman Kodak Co.; 8 from E. I. DuPont de Nemours & Co.; 5 from Calco Chemical Division, American Cyanamid Co.; 3 from Imperial Chemical (Pharmaceuticals) LTD; one from Hartman-Leddon Co. and 9 were synthesized by us.

[3] The carbinol corresponding to 2M2O.

[4] The eight most active anti-lymphoma compounds selected are starred.

Studies concerning the toxicity of the various compounds or the longevity of the treated animals were not included owing to lack of housing space for long term studies of so many animals (442).

Weighed amounts of the compounds equivalent to the concentration needed for a particular test were mixed together with pulverized laboratory chow and then ground in a ball mill for several hours. Test animals were housed separately and given fresh food and water (ad libitum) each day. The various compounds were administered in the concentrations found to have the greatest lymphoma-destroying action and the lowest toxicity for the host; 4M2M and 44MORO in concentrations 0.3 and 0.2 per cent respectively; 4M2OZN, 4M2OCl and 2M2OALC in 0.02 to 0.05 per cent; 4E2O, 4M2O and 2M2O in concentrations of 0.02 and 0.03 per cent.

Treatment of the lymphoma-bearing rats was discontinued one or two days after they became free from tumor. In a few instances, a metastatic or a recurrent growth developed in a previously negative rat after therapy was discontinued especially in rats treated with 2M2OALC and 4M2OCl. Since complete regression of sarcomas did not occur, treatment of the sarcoma-bearing rats was continued until the end of the test.

Some of the compounds were available in such small amounts that the anti-tumor activity could be tested only on a few rats. Nevertheless, those which proved to have anti-lymphoma activity were included as the results may eventually lead to a better understanding of the chemical structures likely to possess tumor-inhibiting properties.

Results

The results obtained from 442 rats bearing palpable tumors which had been implanted 8 to 10 days earlier are recorded in Table 1 as the averaged weight (in grams) of the number of each type of tumor against which a given compound was tested. For instance, 44MORO was used to treat three rats bearing sarcoma #4, three rats with sarcoma #10, three rats with sarcoma #13 and five rats bearing lymphoma #8. At terminal stage of the untreated tumor-bearing rats, the averaged weights of the three sarcomas #4 in the treated rats was 73 grams; in the 19 intreated rats it was 93 grams, giving as is shown in Table 2 a ratio of 73 to 95. The averaged weight of the three sarcomas #10 in the treated rats was 26g; in the 14 untreated rats 68g (a ratio of 26 to 68). The averaged weight of the three sarcomas #13 in the

TABLE 1
Influence of Oral Administration of Eight Anti-Lymphoma Compounds on the Averaged Weight of Sarcoma in Treated Rats.

Type of palpable tumor in the rats	Compound 44MORO		Compound 4M2OZN		Compound 2M2OALC		Compound 4E2OC1		Compound 4M2M		Compound 4E2O		Compound 4M2O		Compound 2M2O		Untreated rats	
	No. rats fed	Av.wt. their tumors	No. rats fed	Av.wt. their tumors	No. rats fed	Av.wt. their tumors	No. rats fed	Av.wt. their tumors	No. rats fed	Av.wt. their tumors	No. rats fed	Av.wt. their tumors	No. rats fed	Av.wt. their tumors	No. rats fed	Av.wt. their tumors	No. rats	Av.wt. their tumors
Sarcoma #4	3	73g	6	67g	6	49g	7	38g	7	39g	12	30g	8	25g	4	22g	19	93g
Sarcoma #10	3	28g	5	53g	6	27g	5	22g	6	22g	9	23g	7	10g	4	14g	14	68g
Sarcoma #13	3	16g	12	12g	6	13g	10	21g	12	12g	12	12g	8	4g	3	3g	16	20g
Lymphoma #8	5	1½g	26	½g	6	4g*	44	6g*	15	0	30	0	25	0	23	1g	55	42g

* Growths of L#8 which arose in negative rats after therapy was discontinued.

TABLE 2
Comparison of the Averaged Gram Weights of Tumors in Treated and Untreated Rats Sarcoma #4, Sarcoma #10, Rhabdomyosarcoma #13 and Lymphoma #8.

Tumors used	Treated with 44MORO	Treated with 4M2OZN	Treated with 2M2OALC	Treated with 4E2OCl	Treated with 4M2M	Treated with 4E2O	Treated with 4M2O	Treated with 2M2O	Not treated
S#4	73/93	67/93	49/93	38/93	39/93	30/93	25/93	22/93	93
S#10	28/68	53/68	27/68	22/68	22/68	23/69	10/68	14/68	68
S#13	16/20	18/20	13/20	21/20	14/20	12/20	4/20	3/20	20
L#8	1½/42	½/42	4/42	6/42	0/42	0/42	0/42	1/42	42

treated rats was 16g and in the 16 untreated rats it was 20g (a ratio of 16 to 20). Lymphoma #8 regressed completely in three of the five treated rats and grew in the other two giving an averaged weight of 1½g for the five treated rats; the averaged weight of the lymphomas in the 55 untreated rats was 42 grams (a ratio of 1½ to 42).

The results indicate that each of the compounds which brings about absorption of lymphoma #8 also causes retardation of the growth of the three types of sarcomas growing in rats fed a diet containing the compound. The extent of retardation of sarcoma growth was greatest in rats treated with the four styryl quinolines (4M2M, 4E2O, 4M2O and 2M2O) which also exhibited the most pronounced lymphoma-destroying activity.

As is shown in Table 1, when the sarcoma-bearing rats were given a diet containing 4M2M, 4E2O or 4M2O which brought about complete absorption of lymphoma #8, the sarcomas did not regress. They remained small, however, and when their weights were averaged and compared with the averaged weights of the same type of tumor in untreated rats, the resulting ratios (shown in Table 2) were 39 to 93 for sarcoma #4, 22 to 68 for sarcoma #10 and 12 to 20 for sarcoma #13 in rats treated with 4M2M, and even less in rats treated with 4E2O and 4M2O.

The results of the experiments indicate that the extent of retardation of growth of the three types of sarcomas in rats treated with the styryl quinolines, 4M2M, 4E2O and 4M2O was equal to about two-thirds that of the size of the same type of tumor present in untreated rats at terminal stage.

The experiments failed to disclose any compound having anti-tumor properties against any one type of sarcoma. Instead the retardation of growth induced by the various compounds seemed to act on the rate of growth of all three types of sarcomas, resulting in small tumors of three sizes varying from that of #4 to that of #13.

In addition to the experiments recorded in Tables 1 and 2, a few tumor-prevention studies were carried out. In these, the animals were placed on therapy the same day or within two days after implantation of the tumor tissue. Tumors implanted three days earlier were not used in preventive treatment, as it had been found that tumor implants became vascularized and began to invade the surrounding tissue 96 hours after implantation (6). The results of the experiments showed that the early administration of any one of the eight

compounds to sarcoma-implanted rats failed to delay the appearance of palpable sarcomas #4 or #10. On the other hand, the appearance of sarcoma #13, possibly due to its slower rate of growth, did in some instances seem to be delayed especially in rats treated with 4E2OCl, but in no instance was it entirely prevented.

During the many passages through which the neoplasms have been transplanted since the earlier studies (1, 3), sarcoma #10 has become somewhat necrotic while #4 and #13 remained free from necrosis. The growth rate of sarcoma #13 has decreased while that of #10 has increased. The actual weight of lymphoma #8 averaged somewhat less (42g) in these experiments than that (61g) given in earlier studies.

The results of the studies of the anti-tumor properties of the eight chemical substances (Table 1) on the growth of the three types of sarcomas compared with that of lymphoma #8 indicate that some as yet unknown metabolic difference probably exists between lymphomatous and sarcomatous neoplasms. Differences found by Seibert et al. (7, 8, 9) in alcoholic extracts of these tumors and also variations in the glucose level of the blood and urine of rats bearing various neoplasms[4] may eventually help to explain the divergency in response to styryl quinoline compounds exhibited by sarcoma-bearing and lymphoma-bearing rats.

Summary

Studies of the anti-tumor activity of seven styryl quinoline and one carbinol compound indicate that substances of this nature which bring about complete absorption of lymphoma #8 in a high percentage of the treated rats, also cause considerable retardation of the growth of sarcomas in rats. The styryl quinolines which were found to have the greatest sarcoma-retarding activity were 4-(p-dimethylaminostyryl) quinoline methiodide, 4-(p-diethylaminostyryl) quinoline, 4-(p-dimethylaminostyryl) quinoline and 2-(p-dimethylaminostyryl) quinoline. Sarcomas in rats treated with any one of these compounds weighed about one-third as much as the same type of neoplasm in untreated rats at terminal stage. The four compounds which proved to have the greatest sarcoma-retarding properties were the ones, with the exception of 2-(p-dimethylaminostyryl) quinoline, which caused complete regression of lymphoma #8 in all the treated rats.

[5] Studies in progress.

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BOOK REVIEWS

Antimetabolites and Cancer. Edited by C. P. Rhoads, A.A.A.S., Washington. 318 pages.

The book consists of 18 reports and a discussion by the participants in the Symposium. The reports primarily cover the work of the individuals and their coworkers on various aspects of the "cancer problem", ranging from comparisons of various metabolic phases in normal and malignant tissues to clinical experiences with the antimetabolites. The discussion that followed the presentation of papers reflects optimism, pessimism, as well as a "wait and see" attitude to the chemotherapy of malignant growth, particularly in man. Differences in the understanding of the basic concept of the "antimetabolite" action were also evident in some individual reports on the matter and in the discussion that followed. The collected papers give a good idea not so much of the magnitude of the "cancer problem" (this has not been defined as yet, except by the practical physicians concerned with the alleviation of the disease), but of the number, variety, and magnitude of biological, microbiological, chemical, and clinical work in progress that can so easily be discussed as pertinent to the "cancer problem". Perhaps these investigations are indeed pertinent to the "cancer problem", perhaps they are not. This state of affairs reflects the exploratory nature of the present efforts. Optimism in research is always justified from the standpoint of stimulating effort and it should be encouraged as long as the "cancer problem" is in the exploratory phase. Symposia, such as are described in this book, are necessary for purposes of orientation. But, as one of the participants very relevantly stated in the discussion, workers in the cancer field must refrain from prophesying that death from cancer will be checked by chemotherapy in six years, because those who make these statements may be called upon to deliver. Clearly, it appears, the delivery date is not in sight.

J. A. Stekol
Sept. 28, 1955

Perspectives and Horizons in Microbiology. A symposium edited by Selman A. Waksman. Rutgers University Press, New Brunswick, N. J., 1955. 220 p.

A symposium held in connection with the dedication of the Institute for Microbiology at Rutgers University forms the substance of

this book. With the aid of a distinguished group of contributors, an attempt has been made to present some of the aspects of present and future research in the many directions taken by modern microbiology.

The symposium is organized in three main sections. The first, entitled "The Microbe as a Living System" is introduced by an article by C. B. van Niel emphasizing the need for study of mircoorganisms per se, as illustrated by examples of work on microbial structures and the physiological responses of the whole organism: A. Lwoff discusses lysogenic bacteriophage systems; J. Lederberg, recombination methods in microbial genetics; and B. D. Davis, enzymatic studies of bacterial auxotrophy. Part II, entitled "Metabolism of Microorganisms" presents a series of papers showing some of the directions taken by, and methods used in, present studies of microbial biochemistry. H. A. Barker reviews the metabolic approach historically, and discusses some specific problems of the present in a paper that serves as an introduction to this section. The following papers are by J. W. Foster, on molds as metabolic models; W. W. Umbreit, on the significance of microbial systems to general biochemistry; and P. W. Wilson, on biological nitrogen fixation. D. H. Peterson concludes by outlining the application of microbial systems to chemically difficult steroid transformations.

The third part of the book contains a group of less closely related papers under the title: "Microorganisms and Higher Forms of Life." It contains reviews by M. Heidelberger on problems of immunology; by F. H. Horsfall, Jr., on the use of substances to inhibit the reproduction of animal viruses; and by H. Eagle on microbiological research in the field of antibiotics. R. L. Starkey concludes with a discussion of the microbial systems significant in the life of higher plants. An appendix is included, reprinting the addresses of J. W. Jones, S. A. Waksman, and A. J. Kluyver on the occasion of the dedication of the Institute.

The various participants in this symposium have each contributed thoughtful analyses of the direction of research in their respective fields. The result is a volume that should be of interest to a wide variety of readers, and a particularly fitting celebration of the opening of the Institute for Microbiology.

JEROME J. FREED

Biological Specificity and Growth. Edited by Elmer G. Butler. Princeton University Press, N. J., 233 pages, 1955.

The Twelfth Growth Symposium of the Society for the Study of Development and Growth appeared in book form in the spring of 1955. This represents the second symposium of the Society which has been published as a book and, under the editorship of E. G. Butler, continues the fine features which were characteristic of the first. The format and type are excellent. The inclusion of an index, an unusual feature for a symposium volume, helps to unify the diverse topics considered. Even though most of the fields covered in this volume are not "fast moving" ones the two year interval between the symposium and its appearance in print have witnessed some advances which are important for a few of the topics under discussion. The Society has been aware of the need for faster publication of the Symposia and has speeded the publication of the 13th Symposium, which is already available.

Dr. Butler, in his Foreword, keynotes the major theme which runs through this volume. To quote: "When it can be determined that interactions among components of a biological system are such that specific activities occur, or when a particular stimulus, internal or external, evokes a specific response, it becomes of considerable importance to discover a basis for the selectivity and specificity concerned." In line with the title "Biological Specificity and Growth" most of the chapters in this book deal with one or another aspect of specificity in relation to growth and differentiation.

Four of the papers may be considered as more or less of a unit which deals with specificities during embryonic development. Two of these deal with immunological studies during development. Schechtman includes an excellent review of past work in the field. He emphasizes an important aspect of development which is only recently receiving attention. This involves the passage of macromolecules from mother to embryo either via the egg or, in the case of mammals, via the extra-embryonic membranes. The possible implications of this situation are discussed. Woerdeman's discussion of organ antigens serves to complement Schechtman's paper. He has a good summary of work to date. There is little emphasis on the "problems of induction", most of the paper is concerned with the "realization" (reviewer's quotes) of specific protein properties in tissues.

Shen's discourse about the ontogeny of specific proteins (enzymes) emphasizes the paradox which results from the application of precise enzymological procedures to an ill defined and little quantified process —that of "biological differentiation". Even so he provides an excellent account of the relation between enzyme proteins and function in a number of systems. Briggs and King have a very good review of nuclear-cytoplasmic interaction and nuclear differentiation. They stress the point that nucleo-cytoplasmic specificities appear at the time of gastrulation. Nuclear differentiation (as determined by the behavior of nuclei transplanted to an enucleated egg) was still not apparent at the end of gastrulation. Some of their more recent researches have produced evidence that nuclear fixity occurs in some cell types shortly after this stage.

I was struck by the similarities in basic mechanisms which determine mating compatibilities and incompatibilities in plants (Roper) and those involved in establishing innate immunity in host-parasite relations (Taliaferro). This review would be far too lengthy if much more detail were discussed. Both of these papers contain very useful surveys of their topics.

Brown and Robinson discuss the development of qualitative changes during growth of plant roots. They have a neat system in which different segments of the growing root represent different stages. Growth processes are related to and controlled by metabolic patterns (enzymatic) which, at different levels, are involved in the establishment of different protein states. These authors as well as Shen, in his paper, stress the possible dual role of enzymes which seem to function as structural proteins and part of the metabolic machinery. Harrison covers types of reactions of bacteria and protozoa to immune bodies. Some of the mechanisms are discussed in detail. Irwin supplies a thorough discussion of the immunogenetics of blood cell antigens. He has an exciting account of the establishment of mosaic blood types in twin cattle due to the intermingling of their blood cells.

Weiss presents a discussion of his now well known template-antitemplate hypothesis for growth control. Since a number of the experiments from his own laboratory which are important for his argument have still not been published in detail it is unfortunate that Weiss elected to write a condensed account of his talk and did not include much of the data which he presented at Durham. Nevertheless his thesis has wide implications and evokes considerable attention.

Greene's paper on the behavior of tissues as grafts serves to emphasize the fact that incompatibilities may be minimized by proper procedures. He describes the effect of simultaneous transplants of two embryonic organs. Both growth and differentiation are enhanced as compared to single organ transplants. The use of embryonic tissue, which is frequently more reactive than adult structures, in disease work is discussed. The persistence of embryonic tissue under heterologous grafts conditions and the importance of stroma induction for graft establishment (especially in cancer tissue) are some of the other provocative topics with which he deals.

In general the caliber of all of the papers is very high. This book should be of great value to most biologists and especially to graduate students who are looking for an evaluation of some of the most important current thinking in biology.

EDGAR ZWILLING

Acceptable work will be published within three months with no restrictions as to length. To accomplish this, papers must be accompanied by a minimum order for 200 reprints at $3.00 per page of text. Tables, illustrations, and mathematical equations approximately $4.20 per page. Rates for larger orders of reprints will be supplied on request.

All papers must be accompanied by an abstract not exceeding 225 words, for *Biological Abstracts*, and by a running title not in excess of 35 letters. Papers may be written in English, French, German, or Italian.

References should be arranged at the end of the paper in alphabetical order by author, and then numbered. The title must always be given, and the inclusive pages indicated. Following is a correct sample form of journal reference:

12. Doe, J. 1942. The preparation of manuscripts. *Growth*, **6**, 700-725.

If a book, the above sample reference form would be:

12. Doe, J. 1942. The Preparation of Manuscripts. New York: Holt. Pp. 400.

In the text, references should be made to the author and either the year or reference number, e.g., Liebig and Wöhler (1837) or Liebig and Wöhler (12),—or still more exactly, Liebig and Wöhler (12, p. 15). Summary and conclusions should be included; footnotes should be avoided. Changes from copy will be charged to the author.

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